THE DISTRIBUTION OF LACTATE BETWEEN THE CORPUSCLES AND THE PLASMA IN BLOOD.

by

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INTRODUCTION.

Evidence has been accumulating in the laboratories of Henderson and Van Slyke to prove that blood is a physico-chemical system. The distribution of acid and basic ions in general and that of bicarbonate and chloride in particular have been studied in blood under varying conditions. Reduced and oxygenated blood under different conditions were subjected to different pressures of carbon dioxide and the behaviour of the various ions had been studied carefully. The Lactate ion, however, has not received the same attention by the workers in these laboratories. No doubt it is supposed that other acid ions behave like chloride in this respect. So far as the blood in resting subjects is concerned, the importance of chloride in the buffering mechanism is predominant, and the similar behaviour of other acid ions, lactate and phosphate, relatively small, for the amounts present in the blood are only 15 and 10 mg. per 100 cc. respectively as against 300 mg. of chloride. In the case of blood in fatigued subjects, though the phosphate is nearly doubled in concentration, its rôle may not be important; but the lactate which can on occasion increase to 200 mg. per 100 cc. may present a different case.

The amount of lactate present in the blood has been/

being estimated at 20 to 25 mg. per 100 cc. in resting subjects.

Recently H. Owles (1) and Noshi (2) have shown separately that the concentration of lactic acid in blood during rest is 10 to 12 mg. per 100 cc. In the literature (3) it is stated that the ratio of concentration of lactate in corpuscles to concentration of lactate in plasma is 0.60: Noshi gives the value 0.85 before and 0.45 after muscular exercise. He has not accounted for the different ratios obtained, but merely states that the plasma has a higher concentration than the corpuscles. The ratio of the concentration of lactic acid in corpuscles to that in plasma may differ in arterial and venous blood. Hill, Long and Lupton (4) in 1924 noticed that in human blood when the lactic acid has been liberated in the body as a result of muscular exercise the ratio is smaller; the concentration in the corpuscles is only about 43 per cent of that in the plasma. The ratio was not derived by direct estimation of lactic acid in the corpuscles but was calculated from the amounts of lactic acid present in the blood and in the plasma, assuming that the corpuscles make up 40 per cent of the whole blood. The different ratios for lactate arrived at by various workers may have some significance, especially in the light of the well-known phenomenon of the chloride shift. The unequal distribution of the lactate between plasma and corpuscles was not studied further by/

INTRODUCTION.

by Hill, Long and Lupton, and the present work aims at a systematic study of the behaviour of lactate towards the corpuscles. The previous workers did not estimate the amount of lactic acid in the corpuscles directly. In the work presented here, however, this has been done.

Recently Kerr (5) has noticed that the sodium and potassium ions can diffuse through the corpuscles, when they are suspended in Ringer solution; but the diffusion is hindered by the presence of serum. This peculiar property of serum needed to be studied in relation to the lactate ion. Therefore an attempt has been made to study the diffusion of lactate into the corpuscles, when they are suspended in serum and saline containing different amounts of sodium lactate.

In addition, the present work takes into account the behaviour of the lactate in relation to muscle cells.

Reference is often made to "lactate ratio" C/P and the ratio of concentration of lactate in plasma to that in corpuscles, which mean, the ratio of the amount of lactate in 100 gms. of corpuscles to the amount of lactate in 100 gms. of plasma.

ESTIMATION OF LACTIC ACID IN BLOOD.

CLAUSEN'S METHOD modified by Cotonio, Friedemann and Shaffer; without aeration (Meyerhof: private communication to Dr P. Eggleton.)

Both colorimetric and titration methods for the estimation of lactic acid in biological fluids were in practice before the development of the titration method by Clausen in 1922, (6). Colorimetric methods were found to be unsuitable. The titration method of Von Fürth and Charnass was available only for quantities of lactic acid greater than 10 or 20 mg., consequently large quantities of the fluid had to be taken for analysis. Clausen's method was designed to estimate as little lactic acid as 0.2 mg. in one cc. of the fluid. Thus micro methods for the estimation of lactic acid in blood date from 1922. Clausen employed either 50 per cent sulphuric acid or 0.005N. potassium permanganate solution for the oxidation of lactic acid to acetaldehyde. The reaction is carried out in a pyrex glass tube and the acetaldehyde thus formed is distilled, (with the assistance of a current of air), into excess of sodium bisulphite solution to form acetaldehyde sodium bisulphite.

$$CH_3CHO + NaHSO_3 \rightleftharpoons CH_3.CHOH.SO_3Na.$$

The association is practically complete in neutral or acid solution, provided a large excess of bisulphite is/

is used. The excess of bisulphite is neutralised by 0.1N. iodine and the end point is adjusted to a definite blue colour using starch as an indicator. Sufficient saturated sidium bicarbonate solution is then added to discharge the blue colour, and the previously bound sulphite is titrated with accurately standardised 0.005N. iodine solution.

Friedemann, Cotonio, and Shaffer (7) introduced the following modifications:-

(1) A reflux condenser was used to lessen the carriage of water into the receiver.

(2) Manganous sulphate solution in sulphuric acid was introduced to catalyse the oxidation.

(3) The conditions for the titration of the sulphurous acid bound by the acetaldehyde were accurately defined.

All the methods available and the theory of the estimation of lactic acid in blood are excellently reviewed by C.N.H. Long, 1924 (8).

Friedemann and Kendall, 1929 (9) have corroborated the fact that in the case of blood and muscles, (where the interfering substances proteins and sugar can be removed efficiently), the method of estimation of lactic acid, either by Clausen or Friedemann, Cotonic and Shaffer, gives concordant results. They have also found that in high acid concentrations the yield/

yield of acetaldehyde is less, but the yield does not vary within wide limits of acidity of the solution that is being oxidised; one per cent solution of sulphuric acid is supposed to permit 99 per cent oxidation of lactic acid to aldehyde.

Eggleton and Hill, 1928 (10) found that the oxidation of lactic acid by potassium permanganate (Clausen) with Manganous sulphate in sulphuric acid, as a catalyst, (Friedemann, Cotonio and Shaffer) without aeration (Meyerhof) gave a yield of 97.5 per cent of acetaldehyde. The apparatus designed for this purpose consists of a 300 cc. kjeldahl flask with a delivery tube blown one inch below the neck at an angle of 120 degrees carrying a kjeldahl trap; the fluid containing lactic acid with 5 cc. of ten per cent manganous sulphate in 10N. sulphuric acid solution is introduced into this flask for oxidation and distillation of acetaldehyde; the fluid is diluted with water, if necessary, so that the solution that is being oxidised may contain one per cent of sulphuric acid. The flask is fitted with a glass stopper carrying a tap funnel from which potassium permanganate solution, 0.005N., is dropped into the fluid. This arrangement is designed to avoid the need for aeration or for a reflux condenser.

The above method was used in the present work for the estimation of lactic acid in plasma and in corpuscles.

In/

In a comparison of the amounts of lactic acid present, in the corpuscles and in the plasma, certain steps taken, need to be mentioned. The amount of plasma taken for the estimation is usually twice that taken in the case of the corpuscles in order that the bulk of the protein coagulum discarded may be the same. 2 cc. of 40 per cent trichloracetic acid are generally added to 2 cc. of laked blood or plasma for the precipitation of proteins. But in the case of corpuscles they must be laked well and when the fluid is transparent, without any loss of time, 4 cc. of the above acid should be added, thoroughly mixed, and the mixture allowed to stand for ten minutes. It is important that in the case of corpuscles the fluid must be transparent and that there should be a great excess of the acid to secure the precipitation of the proteins. Otherwise the filtrate will have a brown tinge, sometimes not noticed, but this introduces an error in the final end point, as the appearance of the blue colour of iodine with starch is not sharp. Excess of acid does not interfere, as in the next step the acid will be neutralised by sodium hydroxide. In filtering off the proteins the funnel should be covered by a watch glass to avoid errors due to evaporation.

The maximum amount of protein-free filtrate available is taken; although this is a time-consuming step/

step it is found necessary for accurate results, especially when the amount of lactate present in the corpuscles is very small.

The protein-free filtrate is treated with copper sulphate and lime to remove sugar, as described by Van Slyke (11).

The filtrate from the copper-lime is taken with the manganous sulphate-sulphuric acid solution in the kjeldahl flask described above, which is heated by a micro burner: and potassium permanganate solution is added rapidly but not in sufficient amount to produce a permanent pink colour in the solution. The vapours of acetaldehyde are passed through a double surface condenser and collected in a receiver containing 3 cc. of 2 per cent potassium metabisulphite solution.

Direct titration of the bound metabisulphite was carried out with 0.005N. iodine using starch as indicator.

RECOVERY OF LACTATE.

A solution of zinc lactate was prepared by dissolving 0.2 gm. in 1000 cc. of water. 2 cc. of the solution were taken and the amount of lactate present was estimated as described above.

An average of all the experiments, Table I, shows a yield of 98 to 99 per cent of the theoretical amount.

TABLE I./

TABLE I. Lactate in mg. per 100 cc.

No. of exps.	Amount of Lactate. Theoretical	Estimated	Percentage recovered.
8	20	19.8	99
4	20	19.4	97
4	20	20.2	101
2	20	19.2	96
		Average	98.6

The various steps involved in the estimation of lactate in the above process did not interfere with the almost complete recovery of the lactate.

EFFECT OF ANTI-COAGULANTS ON THE RECOVERY OF LACTATE.

The employment of anti-coagulants in the blood has to be taken into consideration. Therefore another series of experiments were carried out to examine the effect of the addition of a mixture of lithium oxalate and sodium fluoride, (in the proportion of 10:1) on the recovery of the lactate from the solution. Different quantities of the above mixture were added to 2 cc. samples of the solution, and the lactate in each of them was estimated. It was found that the presence of the mixture in the solution did not interfere with the recovery of the lactate. The only difference noticed was that it took a larger amount of potassium permanganate solution for oxidation.

TABLE II. Effect of addition of Anti-coagulants.

No. of exps.	Amt. of Soln. & Solid Mixture.	Lactate mg./100 cc	Percentage recovered.
2	2 cc.	19.8	99
8	2 cc. 2 mg.	19.8	99
4	2 cc. 4 mg.	19.6	98
2	2 cc. 6 mg.	19.4	96
		Average	98.5

It may be argued that the presence of the mixture in blood may alter the osmotic pressure in the plasma. To avoid any possible error in this direction, the same quantities of anticoagulant have been used so that the results may be comparable. For human blood 0.10 gm. of the solid oxalate and fluoride mixture had been used per 100 cc. For cats' and rabbits' blood 0.50 gm. per 100 cc. was used and for horses 0.30 gm. per 100 cc. Sometimes defibrinated horse blood was taken, to avoid the employment of an anti-coagulant; and 0.01 gm. per 100 cc. of sodium fluoride added to prevent glycolysis. In later experiments the use of sodium fluoride was discarded because there was no appreciable glycolysis as long as the blood, drawn under aseptic conditions, was preserved in the refrigerator; and trichloracetic acid was added immediately on laking the corpuscles.

The effect of anticoagulant on the recovery of lactate in blood was also studied.

As above, 2 cc. of defibrinated blood were introduced into four different cylinders; into three of these/

these cylinders 2 mg., 4 mg. and 10 mg. respectively of the solid mixture of lithium oxalate and sodium fluoride described above were added xxxxxxxxxxx and the blood analysed as usual.

TABLE III. Effect of Anticoagulant on the Recovery of Blood Lactate.

No. of exps.	Amt. of Blood & Solid Mixture.		Lactate mg./100 cc.	Percentage recovered.
3	2 cc.		15.0	
3	2 cc.	2 mg.	15.0	100
3	2 cc.	4 mg.	14.9	98.5
3	2 cc.	10 mg.	14.9	98.5
3	2 cc.		20.0	
3	2 cc.	2 mg.	20.0	100
3	2 cc.	4 mg.	19.9	99.5
3	2 cc.	10 mg.	19.6	98
			Average	99

These experiments show that the solid mixture of oxalate and fluoride used in the stated amounts, does not interfere with the recovery of lactic acid from blood.

ADSORPTION OF LACTATE BY PROTEIN PRECIPITATES.

The amount of lactate in 2 cc. samples of the same blood to which various amounts of solid oxalate and fluoride mixture is added, does not vary, only showing that the presence of the anticoagulant does not/

not interfere with the amount of lactate recoverable from blood. The percentage of lactate that is actually recovered from blood may or may not be 99 per cent of the amount that is actually present. It is necessary to study what percentage of lactate added to blood can be recovered. The protein precipitates may or may not adsorb lactate; if lactate were adsorbed by the precipitates the error introduced in the estimation of lactate in the corpuscles would be of a greater magnitude than in plasma.

In 1924 Long (8), after reviewing all the methods for estimation of lactic acid then available, preferred Clausen's method, although he could recover only 85 per cent of the lactic acid added to blood. His experiments show that neither in the removal of glucose nor in the titration of the aldehyde lie the sources of loss of lactic acid. He concluded therefore that the sources of loss of lactic acid are: (1) that the proteins precipitated carry down some of the lactic acid adsorbed; (2) that the oxidation of lactic acid to acetaldehyde is not complete. It is not necessary to go again into the loss of lactic acid in the removal of glucose or in the titration of the bound sulphite, nor even into the question of incomplete oxidation, because after the introduction of the catalyst, manganous sulphate in sulphuric acid, by Cotonio,/

Cotonic, Friedemann and Shaffer (6) the oxidation of lactic acid to acetaldehyde is complete.

After the proteins are precipitated by trichloracetic acid from the laked fluid - plasma or corpuscles - a known volume of the filtrate is taken for further analysis. This takes for granted that the lactate is evenly distributed throughout the solution, including the precipitates. As stated above, by taking proportionately more plasma than corpuscles one can be sure of the equal amounts of the protein precipitates discarded in the case of plasma and corpuscles. The fact that the heavy precipitates in the corpuscles do not carry some of the lactate adsorbed may still be disputed. In order to prove that there is no adsorption the following experiments were performed:-

8.5 gm. of corpuscles were taken and laked with 40 cc. of water, and 10 cc. of 80 per cent trichloracetic acid solution were added to precipitate the proteins. 25 cc. of the filtrate were taken for analysis, and the remaining 25 cc. of filtrate and coagulum were made up to 50 cc. 25 cc. of the latter were again taken for analysis, and the remaining 25 cc. made up to 50 cc. This process was repeated five times. As each time the fluid is diluted by an equal volume, the amount of lactate in each extract must be half of the amount present in the previous extract. If the first extract of 25 cc. contains 20 mg., the second extract of 25 cc. must contain 10 mg., provided that the protein precipitates do not adsorb lactate.

TABLE IV./

TABLE IV. Effect of Repeated Dilution on Lactate Content.

	Exp.	Lactate in Mg.					
		1st	2nd	3rd	4th	5th	6th
Actual	I.	18	9.2	4.8	2.7	1.3	
Theoretical		18	9.0	4.5	2.25	1.12	
Actual	II.	11	5.8	2.7	1.3		
Theoretical		11	5.5	2.75	1.33		
Actual	III.	4.9	3.06	2.1	1.4	0.9	0.7
Theoretical		4.9	3.26	2.16	1.44	0.96	0.64

In experiment III., 8.75 cc. of corpuscles were taken and the volume made up to 60 cc. In this case 20 cc. were taken each time for analysis and the remaining 40 cc. of filtrate plus coagulum made up to 60 cc., so that each extract must contain two thirds of the previous extract.

These results show that there is no adsorption of lactate by the protein precipitate.

Therefore it is concluded that Clausen's method, described for the estimation of lactic acid in blood, can be applied to corpuscles, provided the corpuscles are laked well with a sufficient quantity of water, and enough trichloracetic acid is added, and the whole allowed to stand for some time to secure complete precipitation of proteins.

RECOVERY OF LACTATE ADDED TO CORPUSCLES AND PLASMA.

After proving that there is no adsorption of lactate by the proteins, it is necessary to investigate whether lactate added to plasma or corpuscles is recovered completely.

Sodium/

Sodium lactate solutions containing 69 and 76 mg. per cent were prepared, and the amount of lactate present was determined accurately. The concentration of lactate present in the plasma and corpuscles was also estimated. 1, 2 and 3 cc. of the lactate solution were added to 2 cc. of serum and to different amounts of corpuscles accurately weighed. The mixtures of lactate solution and serum, and the mixtures of lactate solution and corpuscles were analysed.

The amount of lactate recovered was compared with the actual amount that was present in the mixtures of serum and lactate solution, and in the mixtures of corpuscles and lactate solution. The agreement of estimated and actual amounts is good.

TABLE V. Recovery of Lactate added to Plasma and Corpuscles.

Lact. Soln.	Serum mg%.	Corp. mg%.	Amounts taken	Recovered mg.	Theoretic. mg.
69%	17	10.7	1 cc. soln. 2 cc. serum	1.03	1.034
			1 cc. soln. 1.19 gm. cor.	0.82	0.817
76%	20	10.7	2 cc. soln. 2 cc. serum	2.00	1.92
			2 cc. soln. 2.74 gm. cor.	1.79	1.804
76%	20	10.7	3 cc. soln. 2 cc. serum	2.65	2.68
			3 cc. soln. 3.15 gm. cor.	2.60	2.59

The amount of lactate that is added either to corpuscles or plasma can be recovered completely by the above method.

GLYCOLYSIS.

To prevent glycolysis taking place in the blood, the addition of sodium fluoride is necessary. A mixture of lithium oxalate, 10 gm., and sodium fluoride, 1 gm., was finely ground, and 0.50 gm., of this mixture per 100 cc. of rabbit's blood used for the prevention of coagulation and glycolysis in the blood. The following experiments were performed in order to see whether the mixture is effective in preventing the glycolysis of blood.

TABLE VI.

Time in mins.	Zero	30	60	90	120	180
Lact. mg./100 cc.						
Exp. I	110		111.8	112		
Exp. II.	89.4		89.4		89	98
Exp. III.	95.1	94.7		95.5		99.6
Exp. IV.	20	19.9	20	20.1		24

These experiments show that no serious amount of glycolysis takes place within the first three hours at any rate. Only a few of the experiments recorded here occupied as much as two hours.

The possibility of glycolysis taking place after the blood is laked was studied.

0.3 gm., of the above solid oxalate and fluoride mixture was used for 100 cc. of horse's blood for the prevention of coagulation and glycolysis. 2 cc. samples of blood were taken and laked; trichloracetic acid/

acid was added to these samples of laked blood at intervals of 1, 2, 5 and 10 minutes; and subsequent analysis for lactate was carried out as usual. No increase in the amount of lactate was found in the last sample; all gave a concentration of 42-43 mg., per 100 cc. Similar experiments were performed on defibrinated horse blood containing 0.01 gm., sodium fluoride per 100 cc. blood; similar results with no increase in the amounts of lactate were obtained.

These experiments were performed to ensure that on laking the corpuscles, there was no glycolysis before the addition of trichloracetic acid; the acid, however, was added to the laked fluid as soon as possible.

CONCENTRATION OF LACTATE IN BLOOD AND ITS DISTRIBUTION BETWEEN PLASMA AND CORPUSCLES.

RABBITS.

The blood from the rabbits was drawn from the veins of the ear. The hair was closely clipped and the ear cleaned. Dilatation of the veins was caused by rubbing the ear with cotton wool dipped in benzol. The marginal vein of the ear was opened with the point of a sharp scalpel, and the blood from the vein was allowed to drop into a tube containing the oxalate and fluoride mixture.

This method of withdrawal of blood obviously causes excitement in the animal and hence the figures for lactate are high.

TABLE VII. Concentration of Lactate in the Blood of Rabbits.

	mg./100 cc.
31. xii. 30	23.2
	26.1
	47.4
	40.2
6. i. 31	57.4
	60.0
	68.0
	70.0
10. i. 31	47.0
	50.0
	45.0
15. i. 31	67.2
	65.4
19. i. 31	79.5
	70.0
	76.8
18. v. 31	63.8
Average	58.0 mg. per 100 cc.

Noshi (12) estimates from 25 to 160 mg. per 100 cc. The wide range is no doubt due to variation in the degree of excitement of the animal.

DISTRIBUTION OF LACTATE BETWEEN PLASMA AND CORPUSCLES.

In order to separate the plasma from corpuscles the blood was centrifuged for one hour at 3500 revolutions per minute. The estimation of lactate in blood, plasma and corpuscles were carried out separately; in some cases the amount present in the corpuscles was calculated from the amount present in whole blood and plasma, using the haematocrit to find out the percentage of corpuscles present in the whole blood. This indirect method for estimation of lactate in corpuscles was adopted to check the direct estimations in corpuscles, and it also served as a useful method of checking the results on whole blood, plasma and corpuscles.

TABLE VIII. Distribution of Lactate in the Blood of Rabbits.

	Lactate in mg. per 100 cc.		
Blood	Plasma	Corpuscles	Corp./Plasma
70	102	38	0.38
93.8	111.4	54.6	0.49
	86.5	40	0.47
77	95.9	48	0.50
	150	87	0.58
81	104.6	53	0.50
116	126	71	0.57
70	90	45	0.50
92	111	56	0.50

TABLE VIII. (continued)

Blood	Plasma	Corpuscles	Corp./Plasma
68	82	45	0.55
47	68	42	0.63
81	99	53	0.53
	39.5	23.5	0.60
	27.3	15.6	0.57
	70	43	0.61
	80	46.5	0.54
	123	75.7	0.60
	112	56	0.50
	82	43	0.54
	123	56	0.46
	63	45	0.71
89.2	93.7	45	0.49
	101.2	55.8	0.55
	102.0	65.8	0.63

From the above Table it can be seen that the lactate contained in the corpuscles is about half that in the plasma.

CATS.

In order to obtain cats' blood, the technique described for drawing blood from the ear veins of the rabbit, was used. As cats do not bleed readily, only a few experiments were undertaken. In order to obtain resting blood, the cat was rendered insensible with a blow on the head, and immediately blood was drawn from one of the jugular veins by the open method. In/

In order to obtain blood of high lactate concentration it was only necessary to allow the animal to struggle, as it does when it is kept in the box, and an attempt was made to section the marginal veins of the ear.

When the animal showed signs of fatigue, it was held in position and bled from the marginal veins of the ear.

TABLE IX. Concentration of Lactate in the Blood of Resting and Fatigued Cats.
Lactate in mg. per 100 cc.

	Resting.	Fatigued.
	24	191.4
	27	192.8
	24	241.5
	15.1	241
	24	241
	13.6	232
Average	20 mg./100 cc.	220 mg./100 cc.

Analysis for plasma and corpuscles for lactate was undertaken to see the distribution of lactate in the blood of cats during rest and fatigue, and the results recorded in Table X.

TABLE X./

TABLE X. Distribution of Lactate in the Blood of Cats.

	Lactate in mg. per 100 cc.		
Blood	Plasma	Corpuscles	Corp./Plasma
10.0	11.9	9.5	0.84
13.6	14.0	11.5	0.80
22.9	23.8	21	0.90
116	126.7	75.7	0.59
69	82	44.6	0.54
	80.8	46.5	0.57
	113.2	61	0.54
	71.1	35.2	0.50
91.5	98.4	77.3	0.83

Of these experiments, Nos. 1, 2 and 3 were performed on blood from resting animals; and 4 to 10 on blood from fatigued animals.

In resting animals the ratio of concentration of lactate in corpuscles to that in plasma is 0.80 to 0.90, when the amount of lactate in the blood is 20 mg. per 100 cc. As the animal goes into a state of fatigue, increasing the concentration of lactate in the whole blood, the ratio of concentration of lactate in corpuscles to that in plasma drops to 0.50. The concentration of lactate in the corpuscles does not rise to the same degree, as ~~does~~ the concentration of lactate in the plasma. This difference in the lactate ratio between the blood of resting and fatigued animals/

animals was not noticed in rabbits; the low ratio 0.50 observed in rabbits' blood may, to some extent, be due to the high concentration of lactate in the whole blood.

It may be observed that the above experiments show that the concentration and distribution of lactate in the blood of resting and fatigued cats are very nearly equal to those values obtained in man, under similar conditions.

HORSE.

The following Table shows the distribution of lactate in resting horse blood.

TABLE XI. Lactate in mg. per 100 gm.

Blood	Serum	Corpuscles	Corp./Serum.
10.3	11.3	8.3	0.73
12.0	13.9	9.1	0.65
13.3	15.9	10.0	0.63
14.9	17.2	10.7	0.62
16.3	18.8	12.5	0.70
22.5	26.3	16.9	0.64
10.1	12.2	8.2	0.68
Average 14		Lactate ratio C/P	0.66

The concentration of lactate in resting horse blood is slightly more than in man. The corpuscles show a concentration 66 per cent of that shewn by the plasma.

M A N.

The blood from one of the veins of the arm was drawn by means of suction, producing as little stasis as possible. 5 or 10 cc. of blood were drawn as required and emptied into tubes containing 5 and 10 mg. of solid oxalate and fluoride mixture respectively.

Values ranging from 15 to 20 mg. of lactic acid per 100 cc. blood are on record for resting man. The lowest value obtained is 8.5 mg. per 100 cc. (subject breathing oxygen) as recorded by Hill, Long and Lupton (13). In resting muscle the amount of lactic acid present varies from 15 to 20 mg. per 100 mg. in the hands of several investigators. My results showed a value of 15 mg. per 100 gm. The reason for such variations is due to the fact that very slight movements tend to the production of lactic acid. Even under ideal conditions of rest physiologists have found a small quantity of lactic acid in muscles, and it seems impossible to prevent the formation of such small quantities. Perhaps to maintain the tone of the muscles there should be a little catabolism of sugar giving rise to lactic acid. Therefore even in resting persons we find lactic acid in their blood, the amount depending on the previous state of the person. Ordinarily when a subject is allowed to rest for a while before drawing his blood, the lactic acid present already drops to

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16 to 20 mg. per 100 cc. But if the blood is drawn early in the morning, after a night's rest, we find as low figures as 9 mg. per 100 cc. Harding Owles (1), carried out his experiments from 9 to 10.30 a.m. on subjects who had had no food since the previous evening and rested for an hour in a deck chair in the laboratory; he obtained as low values as 11.2 and 11.5 mg. per 100 cc. on two subjects; in one case only Hill and others obtained a value of 8.5 mg. per 100 cc. Noshi has recorded xx 9 mg. per 100 cc. It was noticed in the course of the present work that excitement or fear raises the amount of lactic acid in blood; in the case of those who remained in bed but were afraid of the needle it was found 20 mg. per 100 cc. Therefore it is concluded that though the normal resting blood contains about 10 mg. per 100 cc., slight bodily movements or anxiety tend to raise the lactic acid to 15 to 20 mg. per 100 cc., which explains the difference in the values obtained by several investigators.

By drawing blood early in the morning I have obtained as low values as 10 mg. per 100 cc. of blood for lactate in resting subjects. xxxxxxxxxxxxx Where this precaution was not taken, the values for the concentration of lactate in the blood of resting subjects were high, corresponding to the figures generally accepted.

TABLE XIII./

TABLE XII. Concentration and Distribution of lactate in the blood of Resting Subjects.

Subject	Lactate in mg. per 100 cc. Blood	Plasma	Corpusc.	Cor./Pl.	Haematocr. C:P
1. P.E.	19.6	20.9	17.7	0.84	40:60
2. S.D.	19.8	22.2	17.5	0.80	48:52
3. S.D.	19.6	20.4	18.3	0.74	48:52
4. H.D.	19.2	23.9	17.0	0.70	50:50
5. W.	17.5	19.0	15.3	0.80	40:60
6. S.	28.8	30.8	24.7	0.80	36:64
7. E.	28.1	34.4	25.2	0.73	43:57
8. K.	18.8	22.7	18.2	0.80	44:56
9. W.H.	19.2	20.9	17.2	0.82	47:53
10. J.D.	40.0	50.0	30.7	0.60	50:50
11. H.W.	24.5	24.7	23.4	0.95	30:70
12. Mc.	29.6	29.6	29.6	1.00	30:70
13. S.D.	21.4	22.3	15.0	0.70	48:52
14. I.T.	21.5	23.0	19.0	0.83	50:50
15. W.H.	11.7	12.7	9.4	0.74	47:53
16. H.D.	10.8	12.0	9.0	0.74	40:60
17. C.	13.8	14.4	13.0	0.90	40:60
18. A.	13.0	14.0	12.0	0.85	40:60
19. M.	8.1	9.0	8.1	0.90	43:57
20. K.	10.3	11.2	6.6	0.60	44:56
21. S.D.	12.3	14.4	10.6	0.70	50:50
Average of Expt.15-21	11	Lactate ratio C/P.		0.80	

The above concentrations are referred to volumes of cells and of plasma respectively. Had they been referred to weights the C/P ratio would have been 0.75 instead of 0.80.

In/

In Table XII, in experiments 1 to 14 the subjects were asked to sit in a chair for a few minutes, as is usually done, before their blood was drawn. In experiments 15 to 21 the subjects had had no breakfast and rested for an hour in a chair in the laboratory before their blood was drawn. It will be seen that in the latter experiments the resting blood has a concentration of lactate, 10 to 13 mg. per 100 cc.

TABLE XIIa. Lactate in mg. per 100 gm. Resting Subjects.

Subject.	Blood	Plasma	Corpusc.	Cor./Pl.	Haematocr. C:P
22. S.D.	15.2	20.5	10.0	0.50	50:50
23. S.D.	15.7	21.0	10.4	0.50	50:50
24. H.D.	19.6	24.5	14.0	0.57	50:50
25. S.D.	15.4	19.2	10.7	0.56	50:50
Average	**Lactate Ratio C/P**			0.53	

In experiments 22 to 25 the subjects walked in the open air at about 3 miles per hour for thirty minutes and their blood was drawn after they had rested for a few minutes. Reference will be made under "lactate shift" to the lactate ratio C/P thus obtained.

The results of Harding (1) and Noshi (2) and of my own experiments prove conclusively that the concentration of lactate in the blood of resting subjects is 10 to 12 mg. per 100 cc. No doubt it was not recognised/

recognised before, that the blood lactate tends to rise even after breakfast.

The highest figure obtained by Hill, Long and Lupton (13), so far, for blood lactate in fatigued subjects is 204 mg. per 100 cc. The subjects were asked to run as fast as they could before the blood was drawn; in some cases standing running was adopted. In these experiments it is advantageous to have one or two subjects who are willing to cooperate; this is the reason why Hill and others were able to obtain the highest figure recorded in the literature.

TABLE XIII. Blood Lactate in mg./100 gm. Fatigued Subjects.

Subject	Blood	Plasma	Corpusc.	Cor./Pl.	Haematocr. C:P
1. S.D.	81.0	102.5	55.3	0.55	49:51
2. P.E.	90.0	108	70.6	0.65	50:50
3. S.D.	98.0	120	70.6	0.60	49.51
4. P.E.	108.0	136	76.4	0.56	50:50
Average				0.59	
	Lactate ratio C/P			0.55	

It is clear from the results in Tables XII and XIII that the corpuscles have a concentration, 75 per cent of that in the plasma in resting man; under conditions of fatigue the concentration in the corpuscles drops from 75 to 55 per cent of that in the plasma.

As/

As a result of increased concentration of lactic acid in blood, the lactate ratio C/P drops from 0.75 (in resting state) to 0.55 (in fatigued state).

A comparison of the Tables XII and XIIa suggests that the ratio of concentration of lactate in corpuscles to that in plasma depends on the reaction of blood. In the case of subjects who walked in the open air before their blood was drawn, the lactate ratio C/P drops from 0.75 to 0.53. Presumably the blood of the subjects in Table XIIIa is less venous than that of those in Table XIII. It seems that the distribution of lactate between plasma and corpuscles depends on (a) the reaction of blood and (b) the concentration of lactate in the whole blood. A study of the effects of these two factors on the lactate ratio C/P is undertaken in the next two sections.

DISTRIBUTION OF LACTATE IN ANAEMIC PATIENTS.

TABLE XIV. Lactate in mg. per 100 cc.

Blood	Plasma	Corpuscles	Corp./plas.	Haematocrit C:P.	Subject.
22.4	19.4	29	1.5	13:87	P
19.4	19.3	20.8	1.1	29:71	B
13.5	11.0	16.5	1.5	12:88	C
	21.5	23.5	1.05	30:70	C
22.7	23.2	23.6	1.0	14:86	G
33.2	32.2	36.8	1.1	22:73	E

In the case of anaemic blood, where the percentage of corpuscles is as low as 14 to 20 per cent, the corpuscles have a higher concentration of lactate than the plasma. This is an interesting reversal of the normal condition. Davidson and Gulland (14), in their book on Pernicious Anaemia, state that the mean diameter of red blood corpuscles in pernicious anaemia is about 14 larger than the average diameter of the red blood corpuscles in normal people, i.e. 10 per cent larger. There is possibly a connection between these two observations (see later).

LACTATE SHIFT.

Winton and Bayliss (3) in their Human Physiology, give the following concentrations of lactate in mg. per 100 cc. of plasma and corpuscles.

	Plasma	Corpuscles	Corp./Plasma
Minimum	20	12	0.6
Maximum	200	120	0.6

The ratio of concentration of lactate in corpuscles to that in plasma (C/P) was found to be 0.75 during rest and 0.56 after exercise: the values obtained by Noshi (2) are respectively 0.85 and 0.43; whereas according to Winton and Bayliss (3) it is 0.60 in either case.

In table XIIa where the subjects had spent the morning in the open air, the lactate ratio C/P came down to 0.50. It was thought that the reaction of the blood and muscular exercise determine the distribution of lactate between plasma and corpuscles. In the case of cats whose blood was collected by venesection, the distribution is same as in man. The low lactate ratio C/P, in the case of rabbits' blood is assumed to be due to the collection of blood, because as each drop collects on the ear, it is very likely that the blood was oxygenated.

In order to see the effect of carbon dioxide and oxygen, samples of blood were taken in tonometers and equilibrated/

equilibrated with air, and with a mixture of carbon dioxide and hydrogen for 15 minutes. The blood was centrifuged and the concentration of lactate in plasma and corpuscles estimated.

The following Table illustrates the effects of oxygen and carbon dioxide on the distribution of lactate in blood.

TABLE XV. Lactate in mg. per 100 cc.

	Blood	Plasma	Corpusc.	Cor./Pl.	Haematocr. C:P
Air	47	54.9	23.9	0.46	22:78
CO_2+H_2	46.2	50.2	34.7	0.69	24:76
Air	142	185	56.8	0.31	34:66
CO_2+H_2	144.8	164.6	109	0.67	35:65
Air	95.5	123.5	30	0.24	30:70
CO_2+H_2	96.0	107.6	70.4	0.65	31:69
Air	65.2	71.8	46.5	0.65	26.74
CO_2+H_2	65.3	68.9	55	0.81	27.73

[The CO_2, H_2 mixture contained about 20% CO_2, but exact measurements of partial pressure were not made.]

Of these experiments the first three were performed on rabbits' blood, and the last on cat's blood.

Removal of O_2 from the blood, with substitution of CO_2 resulted in a definite shift of lactate from plasma to corpuscles.

THE LACTATE SHIFT IN REDUCED BLOOD.

To distinguish the parts played by oxygen and carbon/

carbon dioxide respectively, it was decided to study the effect of different pressures of carbon dioxide on blood in the absence of oxygen. Hydrogen was chosen to dilute carbon dioxide. Since large quantities of blood are required for this purpose, horse blood was selected. Working on horse blood has another advantage, that it shows very little tendency to spontaneous haemolysis. Two horses were available to supply blood for experiments in this work; blood from the slaughter house was avoided. To avoid introducing errors due to any changes in the osmotic pressure, the use of oxalate and flucride mixture was discarded. Blood was drawn aseptically from a jugular vein by means of a canula, and defibrinated and preserved in the refrigerator. Glycolysis no doubt took place, though to a very small degree. If any glycolysis takes place before the blood is subjected to a mixture of carbon dioxide and hydrogen in the tonometer, it is of no importance for the purpose of the experiment. Glycolysis being an enzyme action, the process must be greatly reduced when the blood is preserved in an ice box.

Samples of blood were taken in tonometers containing different mixtures of carbon dioxide and hydrogen; and the tonometers rotated for 15 minutes for the mixture of gases to come into equilibrium with the blood which formed a thin film in the tonometer. The blood was introduced into the centrifuge tubes under a layer of liquid paraffin and the tubes were closed with rubber stoppers. The blood was centrifuged for one hour at 3500 R.P.M. to separate serum and corpuscles. The lactate in serum and corpuscles was estimated directly.

The/

The following two Tables illustrate the effect of carbon dioxide on reduced blood.

TABLE XVI. Effect of CO_2 on Reduced Blood.

Lactate in mg. per 100 cc.

Pressure of CO_2%	Plasma	Corpuscles	Corp./Plas.	Haematocrit C:P
Nil	32.3	20.7	0.64	34.66
H_2	33.0	20.6	0.63	34.66
Air	37.2	18.8	0.50	33.67
1% CO_2 H_2	46.9	27.7	0.59	34.66
do. "	45.2	27.5	0.60	34.66
1.5 "	46.9	29.0	0.61	35.65
3 "	44.7	29.8	0.66	35.65
7.5 "	40.0	28.1	0.70	35.65
10 "	33.3	30.1	0.90	35.65
12 "	34.0	26.6	0.78	35.65
16 "	32.7	29.4	0.89	35.65
20 "	32.0	30.0	0.90	34.66
Air	72.0	33.6	0.47	
1% CO_2+ H_2	68.8	35.5	0.52	
1 "	71.0	36.9	0.52	
2 "	58.0	35.9	0.62	
3 "	62.1	39.3	0.63	
4 "	54.8	35.3	0.64	
8 "	53.6	37.8	0.69	
8 "	56.0	38.9	0.69	
12 "	49.8	37.7	0.76	
16.8 "	45.6	36.6	0.80	
17 "	45.4	36.8	0.81	
Air	51.8	27.7	0.53	

Although there is slight glycolysis the value of the experiments is not lost, because the ratio of the concentration of lactate in corpuscles to that in serum before and after the glycolysis had taken place is about 0.50, on treating the blood with air. Hydrogen has no effect on the lactate ratio C/P. There is a progressive increase in the concentration of lactate in the corpuscles as the pressure of carbon dioxide is increased.

LACTATE SHIFT IN OXYGENATED BLOOD. The above experiments were repeated using air instead of hydrogen for diluting carbon dioxide. The effect of carbon dioxide on oxygenated blood is shown in the following Table.

TABLE XVII. Effect of CO_2 on Oxygenated Blood.

Lactate in mg. per 100 cc.

Pressure of CO_2 %	Serum	Corpuscles	Corp./Serum
Air	47.0	24.8	0.53
4	45.7	27.9	0.61
6	46.4	29.6	0.63
9	44.5	31.7	0.69
10	43.4	29.4	0.70
11	45.2	32.5	0.72
12	43.0	31.6	0.74
16	41.0	32.8	0.80
20	39.9	34.6	0.87
Air	46.0	23.0	0.50

TABLE XVII. continued.

Pressure of CO_2%	Serum	Corpuscles	Corp./Serum
Air	36.0	18.0	0.50
4	36.6	22.0	0.60
6	34.7	22.4	0.64
8	34.5	23.1	0.67
6	33.5	21.6	0.64
10	33.5	24.0	0.71
10	31.0	22.1	0.71
16	32.9	26.5	0.80
20	30.9	26.7	0.86
Air	31.0	15.3	0.50

These experiments were carried out as quickly as possible after the withdrawal of the blood, in order to avoid glycolysis. The phenomenon of the lactate shift is again observed.

COMPARISON OF THE LACTATE SHIFT IN REDUCED AND OXYGENATED BLOOD.

The effect of carbon dioxide on oxygenated and reduced blood is the same; there is an increased concentration of lactate in the corpuscles. For the same pressure of carbon dioxide, there is a greater proportion of lactate present in the corpuscles of the reduced blood than in the corpuscles of oxygenated blood. The difference in the behaviour of lactate towards/

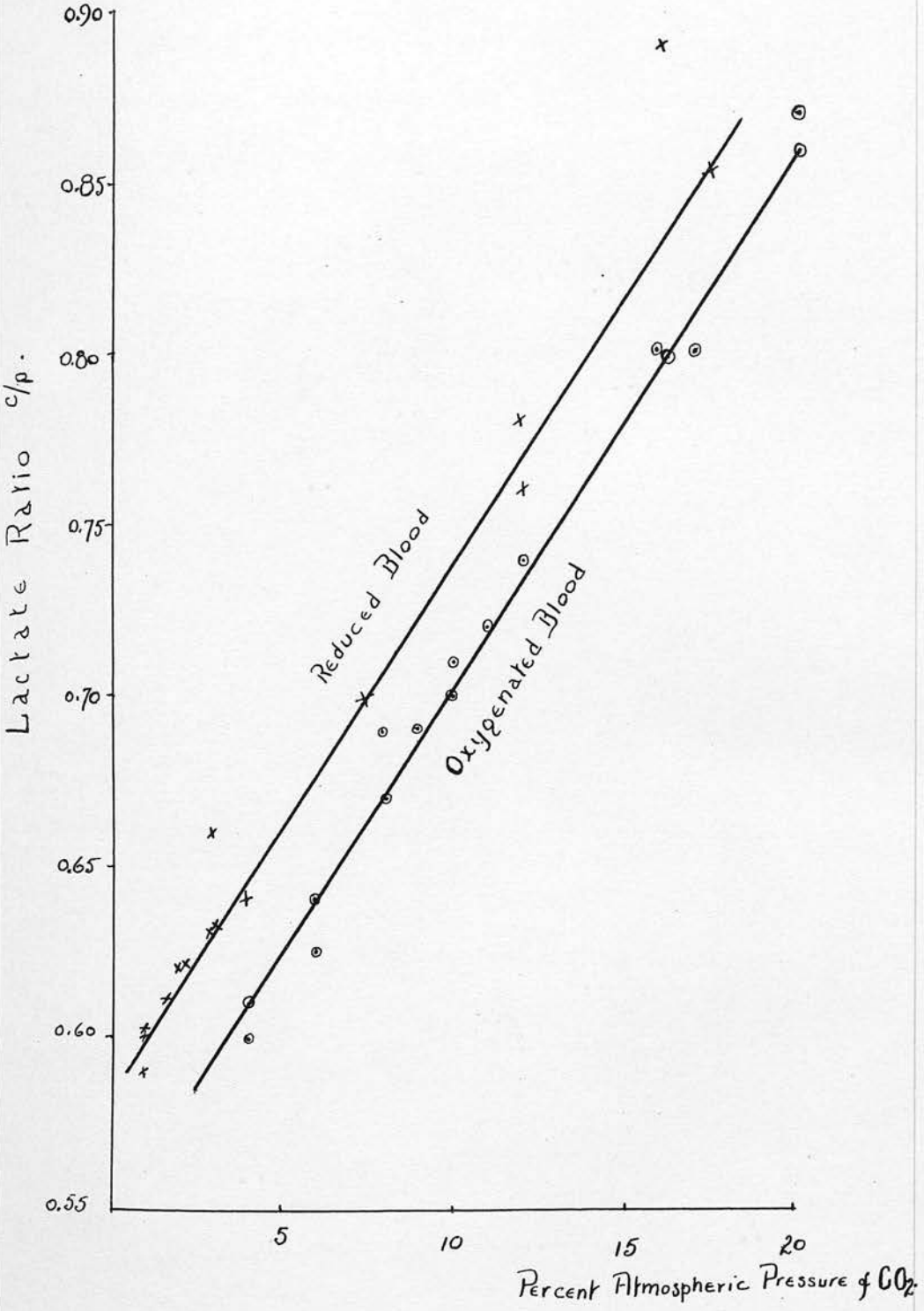


FIG. I.

Lactate Ratio in Reduced and Oxygenated Blood at different pressures of carbon dioxide.

towards oxygenated and reduced blood is similar to the behaviour of chloride studied by Van Slyke, Hastings, Murray and Sendroy (16).

TABLE XVIII. Lactate Ratio in Oxygenated and Reduced Blood.

Pressure of CO_2%	Oxygenated Blood	Reduced Blood
Nil	0.50	0.60
4	0.60	0.64
6	0.64	0.66
8	0.67	0.69
10	0.71	0.73
12	0.74	0.78
16	0.80	0.85

For the same pressure of carbon dioxide the ratio of concentration of lactate in corpuscles to that in plasma for reduced blood is higher than for oxygenated blood (See Fig.I, page 37).

DIFFUSION OF LACTATE INTO CORPUSCLES.

It was noticed that the ratio of the concentration of lactate in corpuscles to that in plasma, is less in blood containing a high concentration of lactate, as a result of exercise, (about 0.5), than xxxxxxxxxxxx in the blood of resting subjects (about 0.75). In other words, as the concentration in the plasma increases, so also does that in the corpuscles, but to a less extent.

The effect of increase in the carbon dixoide pressure is to cause a shift of lactate from the plasma into the corpuscles, (see previous section). Exercise produces a rise in carbon dioxide pressure of the blood and this should result in a similar lactate shift, that is to say, one would expect to find in the blood of fatigued subjects a lactate ratio C/P greater than is found in the same subjects at rest; but the opposite is in fact observed: the lactate ratio C/P falls to a little over half the value characteristic of the resting condition. But the position is obviously complicated by the change in the total quantity of lactate present in the blood. There may be a tenfold increase in the amount of lactate per 100 cc. of blood as a result of exercise.

One might suppose that the corpuscles had not had/

had time to pick up the lactic acid discharged into the blood after a spell of exercise. It is hard to believe this, since the size of the corpuscles is so small that a state of equilibrium between the lactate in plasma and in corpuscles should be reached almost instantaneously. In one instance the subject P.E. continued taking exercise over ten minutes and yet the ratio was found to be low. Kerr (5) is of the opinion that two hours are required for sodium and potassium ions to reach a state of equilibrium, when the cells are suspended in Ringer solution. He admits, however, that this problem was not studied by him. Moreover with each respiratory cycle (i.e. in less than 2 minutes) there is a to and fro chloride shift. However, in order to determine whether there is any delay in arriving at a state of equilibrium between the lactic acid in plasma and in corpuscles, the following experiments in vitro were carried out.

50 cc. of defibrinated horse blood were taken and thoroughly equilibrated with air, to avoid subsequent redistribution of lactate ion due to changes in the gaseous contents of the blood during further manipulations. A suitable quantity of 7% lactic acid solution was well mixed with 50 cc. of blood in a tonometer; 10 cc. of the blood were transferred immediately to a centrifuge tube for separation of serum and corpuscles. The tonometer containing the blood and lactic acid was rotated for another two minutes; another sample of 10 cc. of blood was taken into the centrifuge tube for separation and subsequent estimation of lactate in serum and corpuscles. The process was repeated at nine minutes, thirty minutes and eighty minutes.

TABLE/

TABLE XIX. Time taken to reach Equilibrium on addition of Lactic acid to Blood.

	Lactate in mg. per 100 gm.				
	1 min.	3 min.	9 min.	30 min.	80 min.
Serum	99.2	102	98.2	100	100.1
Corpusc.	48.8	47	51.6	52.7	50.1
Corp./Se.	0.49	0.45	0.52	0.53	0.50

There is no appreciable steady alteration of the lactate ratio C/P with time.

It might be argued that a state of equilibrium could have been reached during the process of centrifuging even though such a state may not have existed at the time of taking the sample of blood for separation of serum and corpuscles. This difficulty may be overcome by omitting the analysis of the corpuscles, and confining the estimation of lactate to serum only. Sufficient serum free from corpuscles can be obtained within 2 or 3 minutes, whereas to obtain corpuscles for analysis, centrifugalisation for 1 hour at 3500 R. per minute is necessary. If the corpuscles take up an amount of lactate proportional to the length of the time they are allowed to remain in contact with the serum, containing a higher percentage of lactate, there should be a progressive diminution in the concentration of lactate in the serum. Two more experiments were performed.

40 cc./

40 cc. of blood were taken in a tonometer, equilibrated with air, and 0.4 cc. of 17 per cent lactic acid was added. The blood was thoroughly mixed, and as above, 10 cc. of the blood were transferred to centrifuge tubes at intervals of 2, 5, 10 and 25 minutes. The blood was centrifuged at the highest speed for two minutes in order to obtain just 2 cc. of serum, free from corpuscles, for analysis.

Concentration of Lactate in Serum in mg./100 gm.

	2 min.	5 min.	10 min.	25 min.
1.	206	205	206	204
2.	204	203	206	205

After the introduction of lactic acid into the blood, suppose it takes 5 minutes for the separation of the first sample of serum, and 30 minutes for the last sample, yet there is no difference in the concentration of lactate in the two samples. (In all these cases the volume of lactic acid added to the whole blood is so minute that there could be no alteration in the percentage of the corpuscles to serum by volume).

It is therefore concluded that a state of equilibrium is reached immediately, at any rate before the blood is drawn from the arm vein of a person who had taken exercise. Eggleton and Evans (17), (using dogs) found that when the muscles of one limb are stimulated it takes three minutes before the same concentration of lactate is found in blood from a stimulated and an unstimulated limb. In this work, in all cases where lactate or lactic acid was added to/

to blood in vitro, about 20 minutes were allowed for the diffusion of lactate before separating the corpuscles from the medium in which they were suspended. Similarly a few minutes were allowed for circulation to be completed, before drawing blood from a vein of a person who had taken exercise.

LACTIC ACID ADDED TO BLOOD.

To study the effect of increased concentration of lactic acid in blood on the ratio of concentration of lactate in corpuscles to that in plasma, suitable quantities of lactic acid solution were added to blood; after mixing, the analysis for lactate in corpuscles and serum was carried out.

Samples of 10 cc. of blood were taken in tonometers and equilibrated with air for 15 minutes; they were allowed to stand for a few minutes for the corpuscles to settle down. 2 cc. samples of serum from blood previously centrifuged were taken in several tubes, and different amounts of lactic acid solution were added. The 2 cc. samples containing various quantities of lactic acid were added to the blood in the tonometers. This procedure of diluting the acid with serum before it is added to the blood, was adopted to prevent the haemolysis of corpuscles which may take place when the undiluted acid comes into direct contact with the latter. This procedure of diluting the acid with serum was abandoned in the third set of experiments, as it altered the percentage of the corpuscles in the blood. In every case 10 cc. of blood were taken and varying amounts up to 0.2 cc. of 5 per cent, 10 per cent and 15 per cent solutions of lactic acid were added. The quantities of lactic acid solution added to 10 cc. blood were so minute, that there could be no alteration in the percentage of corpuscles.

TABLE XX./

TABLE XX. Lactic acid added to Blood.

Haematocrit C:S	Lactate in mg. per 100 cc. Blood	Serum	Corpus.	Corp./Serum
* 35:65		27.8	15.7	0.56
23:77	80	97.9	50.0	0.50
22:78		102.0	34.7	0.34
22:78	114	131.0	55.0	0.42
22:78	173	205.0	65.0	0.40
22:78	195	225.0	95.0	0.40
23:77		107.0	43.6	0.40
23:77	126	153.0	57.3	0.37
.. 35:65		82.0	34.0	0.41
35:65		110.4	40.0	0.36
35:65		215.0	68.7	0.32
35:65		287.0	98.9	0.34
35:65		339.4	115.0	0.34
†25:75	44.7	48.4	33.6	0.70
25:75	73.7	81.6	50.6	0.63
24:76	154	171.8	96.5	0.57

* Analysis of blood after oxygenation.

† " " " before "

.. Blood was not diluted with serum.

In expt. 4 the blood was not oxygenated; the effect of carbon dioxide on lactate ratio C/P is very obvious, being higher than in the case of oxygenated blood.

Average lactate ratio C/S is 0.40 when the concentrations are expressed in mg. per 100 cc. Average lactate ratio C/S is 0.36 when the concentrations are expressed in mg. per 100 gms.

In all except three of these experiments the proportion of corpuscles to serum is different from that in the original blood. But since the acid was diluted each time with 2 cc. of serum, the percentage of corpuscles by volume was kept constant in all cases, - although this percentage of corpuscles was different from that in the original blood. The effect that is produced in the distribution of the lactate between plasma and corpuscles is due to the increased lactic acid, and not tc the alteration in the percentage of the corpuscles. Since all the blood samples were equilibrated with air before and after the addition of acid there could be no alteration in the gaseous contents of the blood during the experiment. All these experiments (except No.4) were performed on oxygenated blood at room temperature. In experiment 4 lactic acid was added to unreduced blood; although there is a drop in the lactate ratio C/P, it is not to the same extent; the presence of carbon dioxide with a concomitant lactate shift is responsible for this higher lactate ratio.

The increase in the concentration of lactate in the corpuscles is less than that in the serum, as the amount of lactate in the whole blood increases. The concentration of lactate in the corpuscles ranges between 33 and 42 per cent of that in the serum. The ratio of the concentration of lactate in corpuscles to that in serum is 0.36.

SODIUM LACTATE ADDED TO BLOOD.

In order to distinguish between the properties of the lactic acid and of the lactate ion, a neutral sodium lactate solution was added to blood, and the diffusion of lactate into the corpuscles was studied. Varying amounts of sodium lactate solution were added to 10 cc. samples of blood without altering (a) the percentage of corpuscles in the whole blood; (b) the osmotic pressure in the system; or (c) the pH. of blood.

The technique adopted for this purpose was as follows: 10 cc. of defibrinated horse blood were taken in a tonometer and equilibrated with air. The blood was transferred to a centrifuge tube and accurately weighed. The blood was centrifuged; one cc. of serum was taken and replaced by one cc. of Ringer Locke solution. The mixture was shaken, centrifuged and analysed for lactate in serum and corpuscles. This would show if there is any change in the ratio of concentration of lactate in corpuscles to that in serum, after one cc. serum is replaced by one cc. Ringer Locke solution. Similarly several 10 cc. samples of blood were taken and in each case one cc. of serum was replaced by varying amounts - 1 cc., 0.8, 0.6, 0.4 cc. of modified Ringer Locke-lactate solution containing about 0.200 gm. per cent of sodium lactate. Enough Ringer Locke solution was added wherever necessary to adjust to the original weight, so that the percentage of corpuscles and the osmotic pressure in the serum might be kept constant.

Modified Ringer Locke-lactate solution was prepared by replacing sodium chloride with an equivalent amount of sodium lactate. A concentrated solution of sodium lactate was prepared from lactic acid and sodium hydroxide at a pH of 7.4 - 7.5. A suitable amount of this sodium lactate solution was mixed with Ringer Locke solution modified, so that after mixing, the Ringer Locke-lactate solution had the same osmotic pressure as Ringer Locke solution.

The contents of the tubes were thoroughly mixed for 20 minutes and centrifuged again as usual to separate serum and corpuscles for subsequent estimation of lactate.

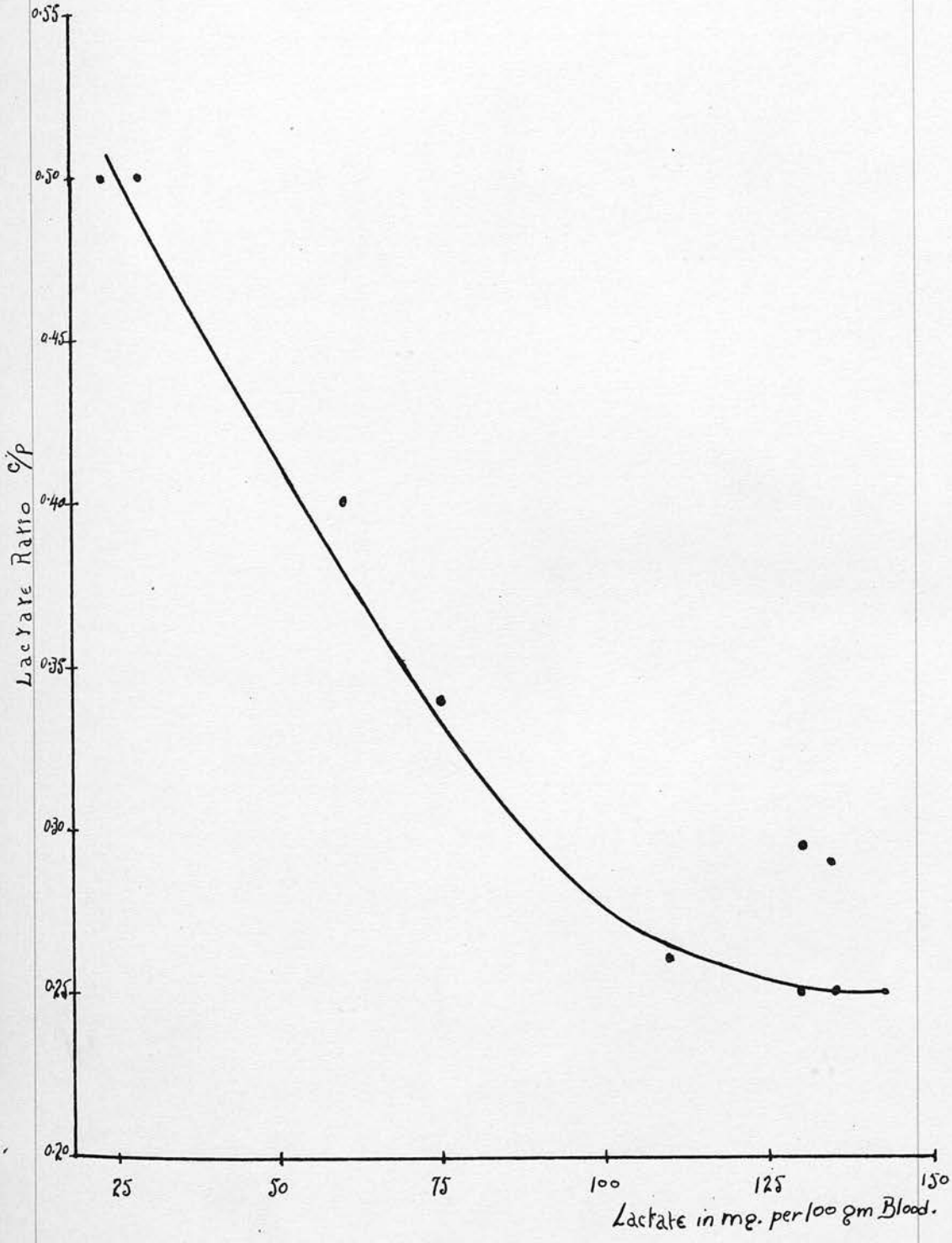


FIG. II.

Lactate Ratio C/P in the blood treated with sodium lactate.

TABLE XXI. Sodium lactate added to Blood.

Lactate in mg. per 100 gm.

	Serum	Corpuscles	Corp./Serum.	Blood.
Original Blood	26.3	16.9	0.64	22.5
" + R	28.5	14.4	0.50	23
" + R-l	215.5	54.6	0.25	151
" + R-l	180.5	54.6	0.29	130
Original Blood	27.4	17.5	0.64	-
" + R	33.0	16.0	0.50	26.2
" + R-l	78.9	31.7	0.40	60
" "	102.0	34.6	0.34	75
" "	141.6	44.6	0.32	125
" "	174.8	50.7	0.29	135
" "	193.6	47.5	0.25	16.3
Original Blood	18.8	12.5	0.70	-
" R-l	185.0	47.2	0.26	110
Average lactate ratio C/P			0.30	

R = Ringer Locke solution.

R-l = Ringer Locke-lactate solution.

Here again the effect of increased lactate concentration in blood is to lower the lactate ratio as in the previous case; the ratio drops steadily from 0.50 to 0.25. (See Fig II, page 47)

The difference in the diffusion of lactate into the corpuscles when (a) lactic acid and (b) sodium lactate was added to blood may be noted. In the case of/

of lactic acid a concentration ratio C/S of 0.36, and in the sodium lactate case a concentration ratio of 0.30 are observed. This difference in the behaviour of cells towards lactic acid and sodium lactate added to blood has to be accounted for; it will be taken up in the discussion.

DIFFUSION OF LACTATE INTO CORPUSCLES SUSPENDED IN RINGER LOCKE SOLUTION.

Kerr (5) has observed that the equilibrium condition between cells and plasma is different from that between cells and Ringer in respect of sodium and potassium. Therefore it was thought necessary to study the equilibrium condition between cells and Ringer in respect of lactate. As before, the percentage of the corpuscles, osmotic pressure, and pH of the solution were kept constant in all the samples of Ringer Locke-lactate solutions in which the corpuscles were suspended.

The corpuscles were not repeatedly washed by Ringer Locke solution. Samples of 10 cc. of blood were taken in centrifuge tubes and weighed. After centrifuging as usual, all the serum was removed very carefully and replaced by exactly the same amount of Ringer Locke-lactate solution, containing suitable amounts of lactate. This was done as in the previous section by adding 1 cc., 0.8, 0.6, 0.4 cc. respectively of/

of Ringer Locke-lactate solution; enough Ringer Locke solution added wherever necessary to adjust to the original weight of the blood. The mixture of corpuscles and Ringer Locke-lactate solution was mixed for 20 minutes. The solution was centrifuged as usual to separate saline and cells.

TABLE XXII. Lactate added to Saline containing Corpuscles.

	Lactate in mg. per 100 gm.		
	Saline	Corpuscles	Corp./Saline
(a)	33.0 (Serum)	16.0	0.50
	150.0	35.6	0.24
	178.9	48.2	0.27
	193.0	49.3	0.25

(a) Analysis of serum and corpuscles in oxygenated blood.

Comparison of the above Table with the figures in Table XXI shows that there is no appreciable difference in the equilibrium of lactate between corpuscles and serum, and corpuscles and saline. In both cases the concentration of lactate in the corpuscles is about 25 per cent of the concentration in the media, serum or saline, (when the concentration in the media is about 193.0 mg. per 100 gms.)

The presence of traces of serum in the saline may be responsible for the similar behaviour of the corpuscles towards lactate dissolved in saline. Therefore it was decided to wash the corpuscles repeatedly with saline.

As/

As usual 10 cc. samples of blood were taken, weighed and centrifuged. The serum was removed and replaced by Ringer Locke solution. The mixture was shaken and centrifuged, and the saline removed. The process was repeated four times. Ringer Locke-lactate solution was then added, maintaining the percentage of corpuscles and the osmotic pressure constant.

The results of analysis of saline and corpuscles for lactate is given below.

TABLE XXIII. Lactate added to Saline containing Corpuscles.

Lactate in mg. per 100 gm.		
Saline	Corpuscles	Corp./Saline
120.0	34.0	0.29
209.0	56.0	0.26
238.5	69.7	0.29
456.7	121.6	0.26

The ratio of the concentrations of lactate in corpuscles to that in saline is the same as in the case of corpuscles and serum. If traces of serum affect the equilibrium they must be very small traces.

Another set of experiments was performed to study the diffusion of lactate from corpuscles into Ringer Locke solution. The technique applied was the same as in the previous experiment, except that lactate was not added. After replacing serum by saline the mixture was allowed to stand from half an hour to two hours for diffusion to take place.

Table XXIV./

TABLE XXIV. Diffusion of lactate from Corpuscles into Saline.

Lactate in mg. per 100 gm.

Saline	Corpuscles	Corp./Saline
8.8	6.3	0.80
10.6	8.7	0.80
9.2	9.0	0.90
8.5	6.2	0.72

In every case the lactate ratio C/P tends to adjust itself to the same value found in resting blood.

LACTIC ACID ADDED TO BLOOD TREATED WITH CO_2.

It had been noticed that on addition of lactic acid to blood in vitro, the corpuscles show a concentration 36 per cent as great as that in the serum (defibrinated blood). In exercise the ratio is higher than the ratio found in vitro. It seemed likely that this was associated with the fact that in the case of exercise, in addition to a high concentration of lactic acid, there is a high pressure of carbon dioxide. Similar conditions were produced in vitro by equilibrating the blood with carbon dioxide at a pressure of 100 mm. Hg., after the addition of lactic acid. A series of experiments were performed: the results are recorded below.

TABLE XXV./

TABLE XXV. Lactic acid added to Blood treated with CO_2.

Lactate in mg. per 100 gm.		
Plasma	Corpuscles	Corp./Plasma
30.2	28.7	0.62
60.5	36.7	0.61
71.5	43.2	0.60
99.8	45.5	0.48
146.2	70.0	0.47
166.4	75.0	0.45
220.9	103.3	0.45

When the blood containing lactic acid to the extent to which it is present in the blood of a fatigued subject, is treated with a high pressure of carbon-dioxide, as is the case in conditions of fatigue, the corpuscles acquire a higher concentration of lactate. i.e., 48 per cent of the concentration in plasma, (instead of 36 per cent when lactic acid is merely added).

EFFECTS OF TEMPERATURE ON THE LACTATE RATIO C/P.

Since the experiments recorded in this paper were all performed at room temperature, on blood from warm blooded animals, it was thought desirable to study the effects of different temperatures on the distribution of lactate between the corpuscles and plasma in blood.

Blood from horses and partially fatigued subjects was taken; in some cases lactic acid was added to horse blood to increase the concentration of lactate. These were thoroughly equilibrated with air before subjecting to different temperatures. 5 cc. samples of blood (horse and fatigued subjects) were introduced into centrifuge tubes kept at temperatures 32°F., 70°F. and 110°F. respectively. The blood was stirred very gently by a glass rod in the tube, at intervals, to prevent the corpuscles from settling down. The centrifuge tubes containing blood were allowed to remain at these temperatures for 20 minutes. The samples of blood were transferred to the centrifuge and revolved at the highest speed to separate plasma from the corpuscles before the blood attained the normal temperature.

TABLE/

TABLE XXVI.

Effects of temperature on lactate ratio C/P

Lactate in mg. per 100 gm.

		32°F.	Room temp.	70°F.	110°F.
Horse	Corp.	8.4		8.1	8.5
	Plasma	16.9		17.6	17.8
	C/P	0.50		0.46	0.48
"	Corp.	8.2			8.3
	Plasma	15.7			15.4
	C/P	0.52			0.53
"	Corp.	87.2	88.1	89.3	91.5
	Plasma	235	244.3	228.7	277.3
	C/P	0.37	0.36	0.39	0.60
"	Corp.	43.5	50.1	51.9	53.2
	Plasma	142	139.2	135.8	139
	C/P	0.31	0.36	0.38	0.38
Subject	T.M.	51.3	46.6	44.5	46.2
		91.3	88.4	93.2	101.4
	C/P	0.59	0.53	0.48	0.46
"	T.A.	76.7	72.4	73.3	
		150.1	166.4	165.4	
		0.51	0.43	0.44	

There/

There is no steady increase in the concentration of lactate in plasma (or corpuscles) with a corresponding decrease in corpuscles (or plasma) to come to any definite conclusion on the lactate ratio C/P as the temperature rises from 30°F. to 110°F. The slight variations in the lactate ratio C/P noticed in resting horse blood and two subjects might be due to alterations in the density, or to a very small amount of glycolysis taking place when the temperature is raised to 110°F. In the case of blood treated with lactic acid, (on account of the high concentration of lactate), slight variations due to changes in density or glycolysis have no effect on the lactate ratio. Therefore variations in temperature seem to have very little effect on the distribution of lactate between the corpuscles and plasma.

INDIFFUSIBLE "PSEUDO LACTATE" IN CORPUSCLES.

The unequal distribution of lactate in the blood of resting and fatigued subjects, suggests that there may be some indiffusible "pseudo lactate" in the corpuscles: and its presence may be taken to account for high ratio in the resting blood and low ratio in the blood of fatigued subjects. The following experiments were performed to study the presence and the amount of indiffusible "pseudo lactate" in the corpuscles.

10 cc. of blood were taken and diluted with 100 to 200 cc. of Ringer Locke solution. After 20 minutes the corpuscles were separated from the saline by centrifugalisation. The concentration of lactate in the corpuscles and in saline were estimated subsequently.

TABLE XXVII.

Effect of Dilution on concentration of lactate in Corpuscles.

	Lactate in mg. per 100 gms.	
Saline.	Corpuscles.	Corpuscles / Saline.
1.5	2.1	1.4
3	4	1.3
1.4	2.0	1.4
1.8	2.04	1.1
1.1	2.8	2.0
1.4	2.0	1.4

If indiffusible lactate is present they must be very small traces.

The concentration of lactate in the corpuscles is greater than that in the saline giving the ratio of concentration of lactate in the corpuscles to that in the plasma more than one. This is not surprising because by interpolating the curve drawn for lactate ratio C/P in the blood to which sodium lactate is added the above result can be obtained.

CONCENTRATION AND DISTRIBUTION OF LACTATE IN BLOOD AFTER GLYCOLYSIS AND EXERCISE.

GLYCOLYSIS. In order to study the effect of a high concentration of lactate on its distribution between plasma and corpuscles, glycolysis was allowed to take place.

Rabbits' or cats' blood was mixed with lithium oxalate to prevent coagulation; no fluoride was added, so that glycolysis might take place, increasing the concentration of lactate in the whole blood. Samples were taken at intervals and centrifuged. The concentration of lactate in plasma and corpuscles was estimated. As in the case of blood to which lactate is added the rise in corpuscle concentration is less than the rise in plasma concentration. It might be argued that this is the result of a more rapid production of lactate in the plasma, but in experiments mentioned in a previous section is was shown that equilibrium between corpuscles and plasma is almost instantaneous.

TABLE 28./

TABLE XXVIII. Effect of Glycolysis on Lactate Ratio.

	Lactate in mg. per 100 cc.		
Animal	Plasma	Corpuscles	Corp./Plasma
Rabbit	99.0	52.9	0.50
do.	208.0	83.0	0.40
Cat 1	71.0	50.0	0.44
do.	113.0	35.2	0.50
Cat 2	91.5	77.3	0.84
do.	106.9	80.5	0.79
do.	113.2	79.0	0.71
Cat 3*	150.0	72.8	0.50
Cat 4*	128.0	55.6	0.43

* No values obtained before glycolysis.

In experiment 1. in Table XXVIII. the ratio of concentration of lactate in corpuscles to that in plasma drops from 0.50 to 0.40. It has been observed already that the ratio in cats' blood is about 0.80, but after glycolysis as low values as 0.71, 0.50 and 0.43 are obtained.

EXERCISE. It has been noticed (in two subjects) that after exercise the ratio of the concentration of lactate in corpuscles to that in plasma drops from 0.75 to 0.55. Increase of lactate in blood in vitro has/

has a similar effect on the concentration of lactate in corpuscles. With a progressive increase of lactate in blood the distribution of lactate between plasma and corpuscles has been studied, by adding lactic acid or sodium lactate to blood. It will be interesting to study the ratio when there is a progressive fall in the concentration of lactic acid in blood. The problem cannot be studied in vitro as we do not know a method for removal of lactic acid from blood. The progressive increase of lactate in vivo has not been studied, because blood has to be drawn at different intervals from the subject who is taking exercise, which is not only unpleasant to the subject but interferes with the exercise; moreover during the interval the concentration of lactic acid in the blood drops, and the sample of blood drawn at a later interval contains more or less the same concentration, in spite of continuing the exercise after the blood is drawn. For this purpose trained subjects, who are willing to co-operate by taking graded exercise, are necessary. As for a progressive fall in the concentration of lactic acid in blood, it is only necessary to draw blood from a vein at intervals after a spell of exercise.

The subject was asked to take exercise either by standing running or running, to increase the concentration of lactic acid in his blood. Blood was drawn from/

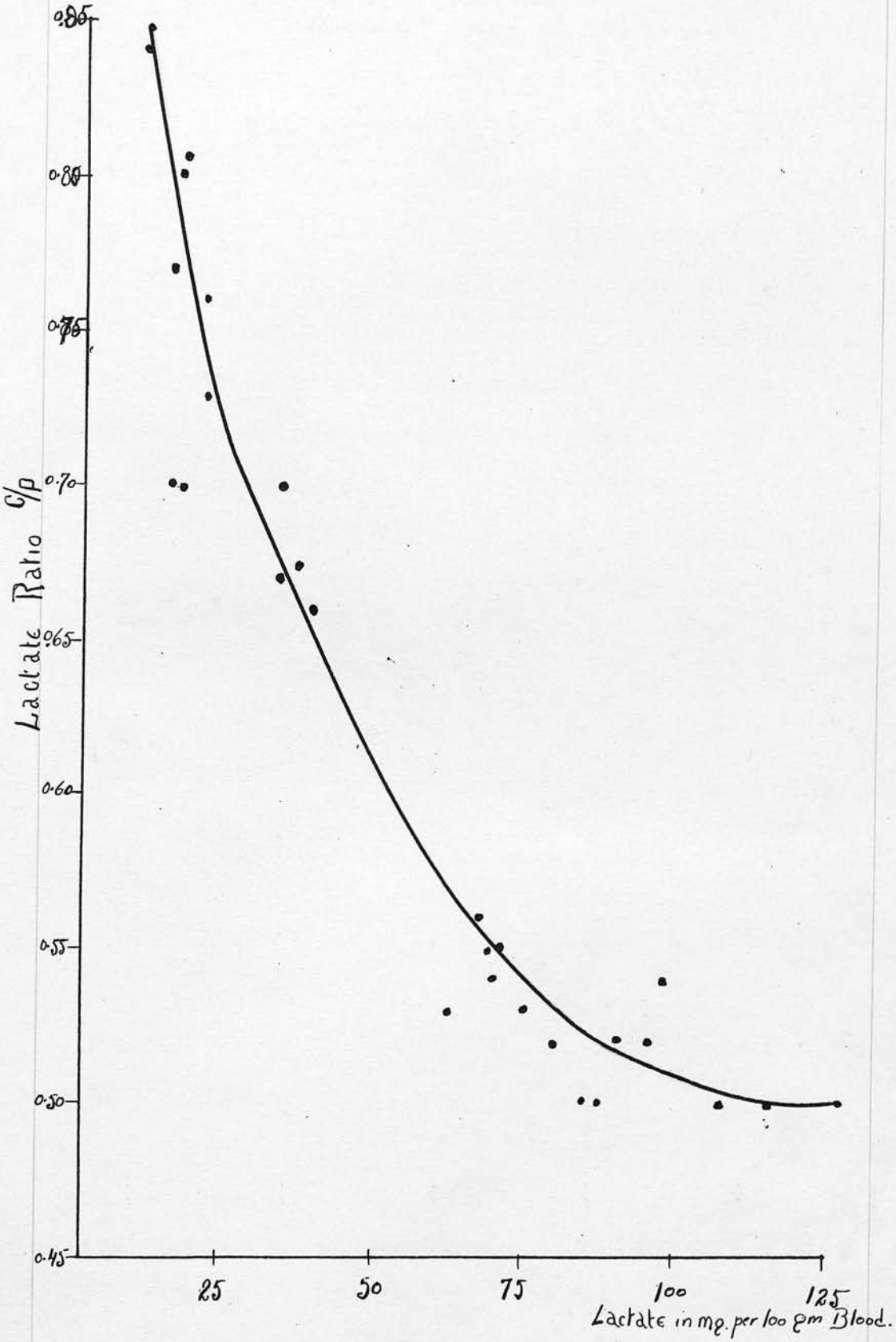


FIG. III.

Lactate Ratio C/P in the blood of subjects before and after exercise.

from one of the suitable veins in the arm at intervals of 3, 7, 20 and 40 minutes. The blood was mixed with oxalate-fluoride mixture (0.1%) and analysed for lactate in corpuscles and plasma. The blood was drawn from an inactive limb; it is therefore assumed that most of the lactic acid that is formed in the muscles is discharged into the blood stream, and that a state of equilibrium in the concentrations of lactate between plasma and corpuscles was reached. In some cases the exercise was completed within a short period; and in other cases it was continued over a period of 10 minutes. The results are recorded in Table 29.

TABLE 29. Effect of Exercise on Lactate Ratio.

Lactate in mg. per 100 gm.					
Blood.	Plasma	Corpuscles	Cor/Pl.	Haematocrit. C:P	Subject.
108	136.0	68.0	0.50		P.E.
90	108.0	56.0	0.52		P.E.
81	102.5	53.3	0.52		S.D.
40	47.3	31.6	0.66	55:45	J.H.
33	37.9	26.5	0.70	56:44	
23.4	26.5	19.4	0.73	56:44	
16.8	18.7	14.4	0.77	58:42	
2.2	24.9	17.8	0.71	59:41	
19.2	20.0	15.0	0.75	60:40	
9.8	120.0	63.6	0.54	50:50	S.D.

2 data

TABLE 29. (Continued)

Blood.	Plasma	Corpuscles	Cor./Pl.	Haematocrit. C:P	Subject.
75.4	101.6	49.2	0.48	50:50	J.H.
71.1	95.3	44.8	0.49	52:48	
35.5	41.6	27.7	0.67	56:44	
18.6	21.2	14.8.	0.70	60:40	
84.6	111.8	55.5	0.50	50:50	S.D.
70.7	90.5	49.9	0.55	51:49	
68.1	86.7	48.7	0.56	52:48	
34.1	40.0	28.0	0.70	51:49	
33.1	36.2	24.1	0.66	34:66	T.M.
35.2	38.4	29.4	0.76	36:64	
16.3	17.0	14.9	0.87	33:67	
114	151.0	76.4	0.50	49:51	D.F.
96	121.0	63.7	0.52	44:56	
63	78.1	41.8	0.54	42:58	
33	38.0	26.0	0.70	40:60	
21.4	24.0	17.0	0.80	38:62	

? Data Hence table arranged

The effects of increased concentration of lactic acid and rise in the pressure of carbon dioxide in blood are both taken into account. The lactate ratio C/P immediately after exercise is 0.50, and as the concentration of lactate decreases in the blood the lactate ratio C/P increases and attains the normal value 0.75. (See Fig III, page 62)

The/

The rise and fall in the percentage of corpuscles is very obvious. During rest it is noted from the haematocrit that the corpuscles form 38 to 40 per cent of the whole blood; and in exercise the corpuscles form from 48 to 50 per cent.

THE DIFFUSION OF LACTATE INTO AND FROM MUSCLE.

Both direct and indirect methods have been employed for determining the concentrations of creatine and orthophosphate in frog muscle, and the same results obtained. The rate of diffusion of various ions into the muscle is different, some attaining a state of equilibrium quickly and others taking a long time.

The diffusion constant of a substance dissolved in the water of a tissue may have a different value from that obtaining in free solution. Eggleton (18) has found that urea and creatine distribute themselves evenly between the water in the muscle and solution; and that no hindrance exists to their free diffusion; from the diffusion of urea into the muscle he was able to calculate the amount of water in the muscle available for diffusion of urea; the figure 80 per cent which he obtained is equal to the amount of water actually present in the muscle by direct estimation. Therefore urea diffuses completely into frog muscle, so that the concentration of urea in the muscle water and the concentration of urea in the solution in which the muscle is suspended are the same. In the case of lactate it was noticed that the diffusion is very slow, especially in fatigued muscle. Nevertheless sufficient diffusion of lactate into and from the muscle takes place when the latter

is suspended in isotonic Ringer solutions containing, respectively, more or less lactate than is present in the muscle. This indirect method for the estimation of the concentration of lactate in frog muscle is specially valuable because during the process of extraction by grinding the muscle with sand in a mortar, the concentration of lactic acid increases unless the muscle is frozen previously, or the process of extraction is carried out at 0°C.

TECHNIQUE. The same procedure that was adopted by Eggleton for determining the concentration of creatine in muscle was adopted. One of the pair of resting gastronemii was placed in a Ringer solution containing, for example, no lactate, and the other in a similar solution containing say 60 mg. per 100 cc. The amount of solution used is the same in each case, about one and a half to two times the weight of the muscle. The gentle stirring of the solution was maintained by bubbling a suitable gas through the solution. The tubes containing the muscle and solution were surrounded by ice in a beaker to prevent the formation of lactic acid; all the experiments were carried out at 0°C. After two to three hours, a known amount of the solution was analysed for lactate. The concentration of lactate in the solutions before diffusion was also determined. As shown by Eggleton the equilibrium concentration of lactate in the muscle can be calculated from the formula:-

$$C = \frac{Lol_1 - L_1 l_o}{(L_o + l_1) - (L_1 + l_o)}$$

C = Equilibrium concentration with muscle in mg./100 gm.

L_o = Initial concentration in high lactate-Ringer

L_1 = Final do.

L_o = Initial concentration in low lactate-Ringer

L_1 = Final do.

According to A.V. Hill(19) the concentration of sodium chloride in a Ringer solution isotonic with resting muscle is 0.71 per cent. This concentration was employed. For fatigued muscle 1.2 per cent of sodium chloride in Ringer solution was found to be isotonic with muscle. The concentrations of potassium chloride and calcium chloride were 0.014 and 0.0125 per cent respectively. In order to keep the pH of the Ringer solution at 7.1, a buffer solution of 0.2 Molar phosphate containing 65 per cent of disodium hydrogen phosphate and 35 per cent of sodium dihydrogen phosphate was prepared; 20 cc. of this solution were added to 980 cc. of Ringer solution. For higher or lower concentrations of lactate, an equivalent amount of sodium chloride in the Ringer solution was replaced by sodium lactate, so that the osmotic pressure of the Ringer solution in high or low lactate solutions is the same.

Sodium lactate at pH 7.1 was prepared by myself. Lactic acid was boiled for eight hours to convert all lactate anhydride into lactic acid. The exact amount of freshly prepared sodium hydroxide solution required for the neutralisation of the lactic acid was determined by a separate experiment using phenol red as the indicator. This amount was added to the acid, and samples were taken and tested for pH 7.1. The amount of acid or hydroxide necessary was separately determined and manipulated until the sodium lactate solution was found to have a pH of 7.1. The introduction of the indicator into the solution was avoided lest it should have any toxic effect on the muscle cells. The diffusion was carried out at 0°C. for two hours in all cases.

RESTING/

RESTING MUSCLE. In order to obtain maximum diffusion muscles of the thigh were chosen. The frog was rendered insensible by a blow on the head or by decerebration, and sectioned immediately above the iliac bones. The abdominal muscles and the viscera in the pelvis were removed, and the skin was carefully dissected off. The legs were separated without injury to the muscles by section of the os pubis with a razor.

Consistent results of 30 mg. per 100 gm. of muscle were obtained for the concentration of lactate in frog muscle. The muscles were placed in a modified Ringer lactate solution containing 30 mg. lactate per 100 cc. solution, and it was found that the muscles were in equilibrium with regard to lactate; the concentration of lactate in the solution remained constant. This figure 30 mg. per 100 gm. of muscle is twice the amount of the accepted figure for resting frog muscle. This was probably due to partial asphyxia of the muscles in the narrow tubes; or due to the dissection of the recti muscles which are attached to the skin; even though the injured fibres were cleared away, there might be fibrillar contractions taking place, giving rise to lactic acid in the muscle. Therefore they were abandoned and gastrocnemii, preferably with their bony attachments, were selected in other cases; the second segment of the leg with the muscles of the calf, were taken. Both these preparations can be made without any injury to the muscles concerned. The muscles were weighed and placed/

placed in one and a half to two times their weight of modified Ringer solution as described above. The actual amounts are given in the Table. The diffusion technique was unaltered. The muscles were removed and drained. They were re-weighed and found to have neither gained nor lost in weight; therefore the solution was isotonic. For the equation given to be valid, there should be no transference of water into or from the muscle. The muscles were stimulated and found to be excitable. 2 cc. of the solution from each tube were taken and analysed for lactate.

An average of the results recorded under experiments 7 to 15 in Table I. shows a concentration of 20 mg. per 100 gm. of muscle. The lowest value obtained is 13 mg. per gm. of muscle.

TABLE 30./

TABLE 30. Concentration of Lactate in Resting Frog Muscle.

Lactate in mg. per 100 gm.

Exp.	Muscle wt. in gm. Initial	Final	Ringer Solution Initial	Final	Equilibr.Conc.
1. 1	10.355	10.350	0	7.2	30
L	10.360	10.355	71.6	61.0	
2. 1	12.485	12.490	0	6.1	32
L	12.495	12.500	67.7	61.0	
3. 1	13.950	13.960	0	5.0	23.6
L	13.870	13.875	67.7	58.4	
4. 1	16.400	16.405	0	3.0	27
L	16.320	16.350	71.0	65.0	
5. 1	16.500	16.510	0	7.7	26
L	15.360	15.350	79.2	63.4	
6. 1	15.975	16.020	0	7.2	29
L	15.945	15.970	37.7	36.0	
7. 1	4.715	4.650	0	6.4	24
L	4.500	4.575	37.7	31.1	
8. 1	4.350	4.355	0	9.9	17
L	4.350	4.355	37.7	26.4	
9. 1	1.368	1.376	0	6.2	24
L	1.395	1.345	44.9	39.7	
10.1	1.834	1.835	0	3.0	13
L	1.845	1.850	37.0	31.5	
11.1	1.225	1.235	0	4.0	21
L	1.230	1.240	37.0	34.0	
12.1	1.450	1.455	0	5.3	18.3
L	1.350	1.240	37.0	31.6	
13.1	0.982	0.985	0	4.5	16.8
L	0.990	0.985	39.4	33.3	
14.1	2.600	2.605	0	3.0	23
L	2.650	2.645	46.0	43.0	
15.1	2.842	2.835	0	2.5	14
L	2.903	2.895	47.2	41.1	

Average of experiments 7 to 15 is 20 mg. per 100 gms.

1 = concentrations of lactate in low lactate-Ringer sol.
L = concentration of lactate in high lactate-Ringer sol.

Experiments 1-7 were performed on thigh muscles.
" 7-15 on gastrocuemii.

TABLE 31. Concentration of Lactate in Fatigued Frog Muscle.

Lactate in mg. per 100 cc.

Exp.		Muscle wt. in gm. Initial	Final	Ringer Solution Initial	Final	Equilibr. Conc.
1.	1			0	22.5	232
	L			512	487.0	
2.	1			0	25.0	220
	L			500	470.0	
3.	1			0	23.6	230
	L			502	476.0	
4.	1	1.245	1.250	0	16.6	270
	L	1.250	1.255	502	489.0	
5.	1	1.625	1.628	0	14.2	214
	L	1.640	1.635	412.7	399.5	
6.	1	1.365	1.390	0	24.5	218
	L	1.375	1.365	412.7	391.2	
7.	1	0.920	0.960	11.0	34.7	214
	L	0.900	0.900	418.0	394.5	
8.	1	1.438	1.445	11.0	30.6	205
	L	1.501	1.495	418.0	396.7	
9.	1	1.095	1.100	11.0	36.0	286
	L	1.090	1.095	418.0	406.0	

Average equilibrium concentration is 238 mg. per 100 gms.

FATIGUED MUSCLE. In this case the calf muscles of the frog were fatigued by causing them to contract isometrically. They were dissected out, weighed and introduced into the tubes containing modified Ringer-lactate solutions. In order to prevent oxidative recovery the solution was covered with a layer of liquid paraffin. The steady gentle stirring of the solution was maintained by bubbling nitrogen through the solution. The use of liquid paraffin renders it impossible to find if the weight of the muscle had remained constant during the experiment; but the muscles could be tested to see if they were still excitable. Later on the use of liquid paraffin was given up and the tubes were stoppered, after the air had been displaced by nitrogen; the current of nitrogen was maintained. The weight of the muscles in these cases was noted and found to be constant. At the conclusion of the experiments the muscles were found to be still excitable. (Results in table 31, page 72).

The results given in Tables 30 and 31 indicate that the lactate dissolved in both resting and fatigued muscles is in simple osmotic equilibrium with the surrounding fluid, since the above equilibrium experiments give the same values as direct estimation recorded in the literature. This condition is in sharp contrast to the conditions obtaining in the system blood corpuscle-saline or in blood itself.

DISCUSSION.

Henderson, Van Slyke and others have expressed the concentrations of acid ions in millimoles in 100 cc. of water in plasma and in corpuscles. In the present work, the concentrations of lactate are expressed in mg. per 100 cc. (or 100 gms.) of whole plasma and corpuscles. A chemist is interested in the concentration of the ions in relation to their osmotic pressure, but it is also interesting to compare the values in mg. per 100 gm. (or 100 cc.) of whole plasma and of corpuscles. The object of this work being to compare the ratio of the concentration of lactate in corpuscles to that in plasma, as long as the results are expressed in one way, it does not matter which method is adopted. The lactate ratio C/P in this work are obtained by expressing the concentrations of lactate in mg. per 100 gm. of plasma and of corpuscles.

DIFFUSION OF LACTATE.

The difference in the rise of concentration of lactate in corpuscles, on the addition of sodium lactate and lactic acid to blood, is noted already; the corpuscles having a higher concentration of lactate when lactic acid is added to blood. Perhaps, sodium lactate being a neutral salt, the ratio of concentration of lactate in corpuscles to that in plasma is 0.30; here it is a matter of simple diffusion of lactate ion into the corpuscles,/

corpuscles. When lactic acid is added, the properties of lactic acid and of lactate ion affect the concentration of lactate in the corpuscles; the acid is buffered by the bicarbonates and proteins in plasma and cells.

Plasma:

$$H\,L + NaH\,CO_3 \rightarrow Na\,L + H_2CO_3$$

$$H\,L + NaPr \rightarrow Na\,L + H\,Pr$$

Cells:

$$H\,L + KH\,CO_3 \rightarrow K\,L + H_2CO_3$$

$$H\,L + KHbO_2 \rightarrow K\,L + HHbO_2$$

Sodium bicarbonate and proteins in plasma on one side and potassium bicarbonate and haemoglobin on the other side compete for lactate. Since the latter are enclosed within the corpuscular membrane the concentration of lactate in the corpuscles depends on the diffusion of undissociated acid into the corpuscles. The reaction $H\,L \rightarrow H^{\cdot} + L'$ goes forward, as the lactate ion combines with the labile base in the plasma. The concentration of lactate in cells will, therefore, depend on the difference in the rate of diffusion of undissociated acid into the cells and of the formation of lactate in the plasma.

In addition to the diffusion of lactate ion, which gives a concentration of 30 per cent of that in plasma, there is a 6 per cent increase due to the buffer mechanism on the addition of lactic acid to blood.

SELECTIVE PROPERTY OF SERUM.

The selective property of serum noticed by Kerr (5) is not observed with regard to the diffusion of lactate into the corpuscles. The ratio of concentration of lactate in corpuscles to the concentration of lactate in serum or saline is 0.27 - 0.30. The equilibrium condition between cells and serum is the same as that between cells and saline. Normally neither the sodium nor the potassium ion diffuses through the corpuscular membrane; the results of Kerr therefore cannot be compared with the diffusible ion, like lactate.

The diffusion of lactate from the corpuscles into saline has been studied and no peculiar behaviour of serum or corpuscles was observed. The amount of lactate in the corpuscles being small, the ratio of concentration of lactate in corpuscles to that in saline corresponds to the value obtained in the blood of resting subjects.

VARIATIONS IN THE RATIOS IN RESTING & FATIGUED BLOOD.

The lactate ratio C/P drops from 0.75 (in resting state) to 0.50 (in fatigued condition). Addition of lactic acid to blood also brings down the ratio to 0.36.

Suppose that the distribution of lactate ion between the water in plasma and the water in corpuscles is equal. In 100 cc. of plasma there are 93 cc. water, and in corpuscles 65 cc. water. The ratio of the amount of water in 100 cc. corpuscles to that in 100 cc. plasma is 0.70. The corpuscles therefore should have a concentration of 70 per cent of that in plasma. All the diffusible ions should have the same ratio if the above supposition were correct; since they do not, the actual conditions cannot be so simple. Van Slyke, Hastings, Murray and Sendroy (16) have made use of a less simple relationship (the coefficient of relative activity) for chloride and bicarbonate ions; they have established the relation between these ions by dividing each $\frac{[H']_s}{[H']_c}$ value by $\frac{[Cl']}{[Cl']_s}$ and $\frac{[HCO_3']}{[HCO_3']}$ value for the same pH and degree of oxygenation. A similar value the coefficient of relative activity for lactate ion showing its relation to other diffusible ions is desirable.

After/

After strenuous exercise, (in addition to an increase in the concentration of lactic acid, together with a rise in the pressure of carbon dioxide in blood), there is also about 10 per cent increase in the volume of the corpuscles. Owing to the rapid circulation, some of the corpuscles stored in the spleen escape into the general circulation; this may alter the concentration of lactate in blood but on physico-chemical basis the lactate ratio C/P should not alter.

It has been noticed that in anaemia, where the percentage of corpuscles is low, the concentration of lactate in cells is higher than in plasma. The converse may not hold good because the amount of haemoglobin in the blood cells of resting subjects and in the cells of fatigued subjects is not different. In other words colour index is not altered. As there was no increase in the percentage of cells in vitro experiments (where low values for lactate ratio were observed), this at any rate may not be responsible for the decrease in the concentration of lactate in cells.

Bock, Dill, Hurxthall, Lawrence, Coolidge, Dailey and Henderson (20) state that in exercise the water in serum suffers diminution to a slight extent, followed by a rise in serum proteins without any change in the concentration/

concentration of solids in the corpuscles; the concentration of base in the serum is found to increase from 154 millimoles to 160 millimoles during exercise. Factors like the increase in serum proteins may be followed by a rise in the concentration of lactate in serum due to increase in buffers.

Loss of water in serum, and increase in the concentration of lactate and other diffusible ions after exercise are bound to alter the osmotic pressure. It is found that the resting frog muscle is isotonic with the saline containing a concentration of 0.71 gm. sodium chloride per 100 cc. water, whereas the fatigued muscle is isotonic with saline containing a concentration of 1.2 gm. sodium chloride per 100 cc. water. In conditions of fatigue the muscle cells are isotonic with saline containing a higher concentration of sodium chloride; by analogy it may be supposed that corpuscles from blood of fatigued subjects are isotonic with plasma containing a relatively higher percentage of salts than that in conditions of rest; lactate also contributes in raising the osmotic pressure in plasma.

The above workers (20) have noticed that the bicarbonate and chloride are increased in cells of blood from fatigued subjects: they state that the increase/

increase in lactate is more than equivalent to the decrease in bicarbonate; there is also a 7 per cent increase in the concentration of bicarbonate in cells and about 10 per cent in the concentration of chloride in cells. The concentration of lactate in corpuscles also increases, but not to the same estent as in plasma. The increase in the concentration of chloride and bicarbonate in the cells may account for the comparatively less increase in the concentration of lactate in the cells of blood of fatigued subjects. Therefore it is not surprising that a low lactate C/P ratio is found in the blood after exercise.

Since the lactic acid is buffered by the bicarbonates, there may be a relation between the relative increase in the concentration of bicarbonate and the relative decrease in the concentration of lactate in cells. It will be interesting to study the lactate ratio in relation to bicarbonate ratio.

LACTATE SHIFT.

So far all the experiments indicate that the corpuscles acquire a higher proportion of lactate when the blood is equilibrated with a high pressure of carbon dioxide. In this respect the behaviour of lactate is similar to that of chloride, accompanied by a transference of water from plasma into corpuscles. There is no need to go into the details of the well-known chloride shift or Hamburger effect. The discovery of the increase in the alkalinity of serum [A. Schmidt, (1867) and N. Zuntz, (1867) independently of one another], and of the decrease in chloride of the serum accompanied by a swelling of the cells (Hermann Nasse, (1874)) when the blood is treated with high pressures of carbon dioxide are recorded by J. Warburg (21). Van Slyke, Wu, and McLean (15) have dealt with this subject and have shown clearly with the aid of diagrams both the chloride shift and the transference of water into the corpuscles.

Besides chloride, lactate must also be taken into account in considering the buffer mechanism of blood. It would seem that the lactate shift is important in blood in conditions of fatigue. The concentration of chloride in blood is nearly constant, and its role in the arterial and the venous blood in/

in the resting state is recognised. In the fatigued condition, the oxygen pressure in the tissue cells falls, and the carbon dioxide pressure increases; the increase in carbon dioxide pressure is also brought about by the action of lactic acid on the bicarbonates in the blood.

The effect of increased carbon dioxide pressure in the blood is to displace sodium and potassium from combination with serum proteins, haemoglobin, and form the bicarbonates of sodium and potassium.

(1) $H_2CO_3 + NaPr \longrightarrow NaHCO_3 + HPr$ (in plasma)

(2) $H_2CO_3 + K\ Hb \longrightarrow KHCO_3 + H\ Hb$ (in cells)

As the amount of base released by haemoglobin (equation 2) is about ten times the amount of base released by the plasma proteins (equation 1), the concentration of bicarbonate in corpuscles will increase to that extent.

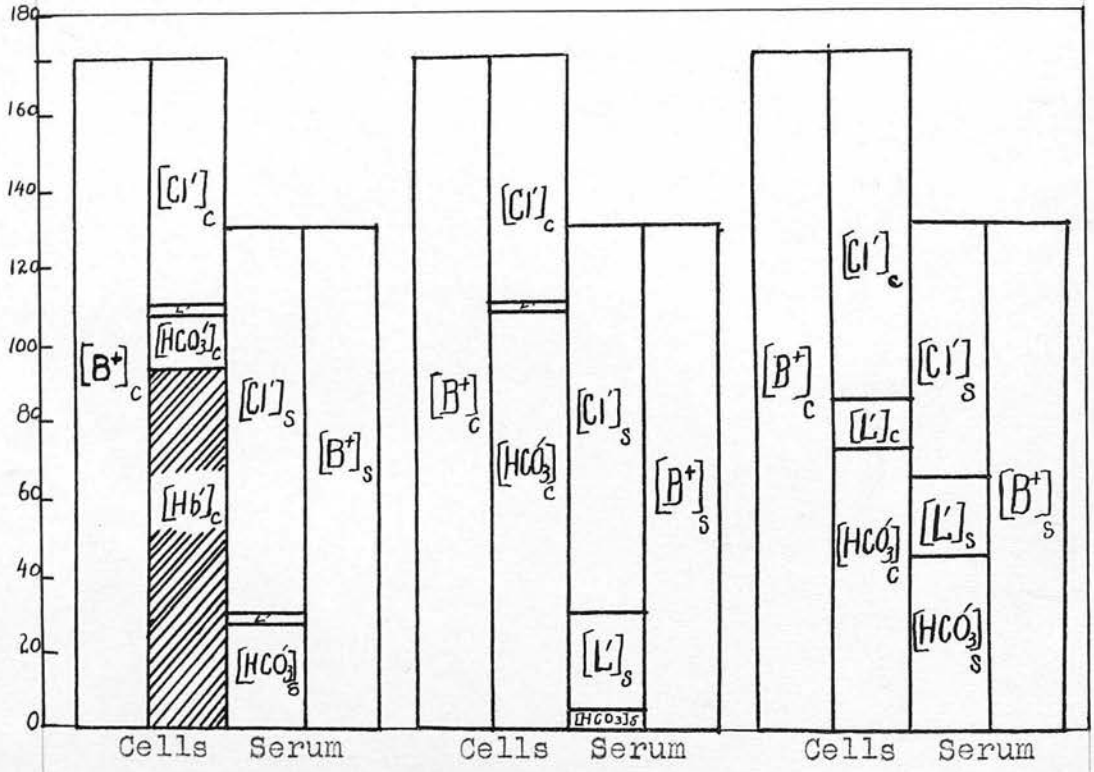
The presence of lactic acid in blood after exercise will result in an increased concentration of lactate in plasma, with a corresponding decrease of bicarbonate - because a large proportion of lactic acid will be neutralised by the bicarbonates in the plasma as shown below.

(a) $H\ L + NaHCO_3 \rightarrow NaL + H_2\ CO_3$ (in plasma)

(b) $H\ L + KHCO_3 \rightarrow K\ L + H_2\ CO_3$ (in cells)

the formation of NaL in plasma (a) being greater than that of KL in cells (b) because the undissociated acid HL has to pass through the corpuscular membrane before the reaction (b) can take place.

The/



BHb replaced in cells by $BHCO_3$

Shift of Cl' and Lactate' to cells and HCO_3' to serum to restore equilibrium.

Fig. IV
pH_s 7.8
Resting blood

Fig. V
After exercise increasing pressure of CO_2 and Lactate.

Fig. VI
pH_s 6.6.

Concentrations are expressed in millimoles per 100 cc. of water in cells and serum.

(Modification of the diagrams as per Van Slyke, Wu and McLean; redistribution of water to restore osmotic equilibrium is not shown.)

The combined effects of (2) and (a) will result in a high concentrate of bicarbonate in corpuscles and of lactate in plasma (c.f. figs. 4 and 5). Now in order to restore the equilibrium an exchange of the lactate in the plasma for the bicarbonate in the corpuscles must take place (c.f. figs. 5 and 6).

If the lactate in the plasma is not exchanged for the bicarbonate in the corpuscles as argued above, the corpuscles must have a low concentration of lactate, - the amount of lactate depending on the diffusion of lactate into the corpuscles. The lactate ratio C/P must be 0.36 after exercise. As a matter of fact in in vitro experiments the ratio of the concentration of lactate in corpuscles to that in plasma is (0.36) lower than that found in partially fatigued animals' blood (0.50): the reason being that in in vivo there is increased tension of carbon dioxide giving rise to lactate shift into the corpuscles. But when the blood treated with a high concentration of lactic acid in vitro is subjected to a pressure of 80 mm. of Hg. carbon dioxide, the concentration of lactate in corpuscles rises; so that the lactate ratio C/P in vitro experiments corresponds to the lactate ratio C/P obtained in vivo experiments.

Therefore it is concluded that the lactate shift into the corpuscles is responsible for maintaining the lactate ratio C/P at 0.50 in the blood of fatigued subjects,/

subjects, otherwise the lactate ratio C/P would have been 0.36. The rôle of lactate in fatigued blood, where the concentration of lactate may be raised considerably, is important, since were it not for the transference of lactate into the corpuscles (under the influence of a high pressure of carbon dioxide as a result of exercise) the concentration of lactate in plasma and that of bicarbonate in the corpuscles would be considerably higher; but by an exchange of lactate for bicarbonate in the corpuscles, equilibrium is maintained.

It may be argued that the lactate ratio 0.50 observed in the blood of fatigued subjects is due to the increase in the osmotic pressure, when lactic acid from the muscles is discharged into the blood. It is precisely for the same reason that in the experiments with sodium lactate, the osmotic pressure was kept constant, the lactate ratio C/P being 0.29; but when lactic acid was added, no attention was paid to osmotic pressure; suitable quantities of 7 per cent and 17 per cent of lactic acid solutions were added to blood to simulate the condition in blood except the rise of carbon dioxide pressure; the lactate ratio C/P observed in this case is only 0.36.

As stated already the phenomenon of the lactate shift accounts for the low lactate ratio C/P obtained for rabbits' blood. The blood collected by drops in

rabbits/

rabbits was oxygenated in the process of collection, hence the ratio is low. Whereas a higher lactate ratio C/P is obtained in the case of blood drawn by venesection in animals, cats and horses.

In arterial blood the concentration of lactate in corpuscles is about 50 per cent of the concentration of lactate in plasma; and in venous blood it is 75 per cent, the concentration of lactate in whole blood being about 20 mg. per 100 cc. This difference in lactate ratio C/P in venous and arterial blood depends on the concentration of lactic acid in whole blood. When the concentration of lactic acid in blood is as low as 10 to 20 mg. per 100 cc. the phenomenon of lactate shift in blood is predominant. But as the concentration of lactic acid increases, the effect of this phenomenon on lactate ratio C/P becomes less and less marked,and accordingly the difference between the lactate ratio C/P in oxygenated and reduced blood gradually falls, as the ratio now mainly depends on the diffusion of lactate ion (diffusion being 36 per cent and the lactate shift 14 per cent).

As the lactate shift helps to prevent the lactate ratio from dropping to 0.30 or 0.36, (as observed in high concentrations of lactate and lactic acid respectively, in blood), it is easy to see why Winton and Bayliss obtained the same ratio for blood containing minimum and maximum amounts of lactate.

Van Slyke, Wu and McLean (15) had predicted in 1923 that the ratio of the concentration of chloride in corpuscles to that in plasma would be higher in reduced blood than in oxygenated blood. In 1925 Van Slyke, Hastings, Murray and Sendroy (16) studied the distribution of diffusible ions between serum and cells of the blood of horse over a range of pH 7.0 to 7.6 in oxygenated and reduced blood. Though the actual values obtained in 1925 for chloride and bicarbonate ratios did not tally with the figures predicted in 1923, the curves of the ratio plotted against pH for both oxygenated and reduced blood have the same slope as the predicted values; the curve representing the ratio for reduced blood is higher than the curve for oxygenated blood. Similarly the concentration of lactate in corpuscles of reduced blood is higher than the concentration of lactate in corpuscles of oxygenated blood, the pH pressure of carbon dioxide in both cases being the same. Table XVIII shows that the lactate ratio C/P for reduced blood is higher than that for oxygenated blood for the same carbon dioxide pressures.

The behaviour of reduced blood is not surprising, - since oxyhaemoglobin is relatively a less weak acid than haemoglobin. Both oxyhaemoglobin and reduced haemoglobin act as buffers, but the value of the latter's dissociation constant renders it a slightly more efficient buffer at the reaction (pH 7.3 to 7.4) of blood.

RÔLE OF LACTATE IN THE RESPIRATORY CYCLE.

Suppose the concentration of lactate in blood is 20 mg. per 100 gms. As the lactate ratio C/P in venous blood is 0.75 the concentration of lactate in plasma and corpuscles will be 22.2 and 16.6 mg. per 100 gms. In 100 gms of blood the amounts of lactate present in plasma and in corpuscles will be 13.4 and 6.6 mg. respectively, assuming that the corpuscles make up 40 per cent. of the whole blood. Similarly in the arterial blood, as the lactate ratio C/P is 0.50, the amounts of lactate present in plasma and in corpuscles will be 15 and 5 mg. respectively. Therefore in the respiratory cycle there is a to and fro lactate shift of about 1.5 mg. per 100 gms. of blood. i.e. 7.5 per cent of the amount of lactate present in blood.

In the case of fatigued subjects suppose the blood has a concentration of 200 mg. per 100 gms. As the lactate ratio C/P in venous blood is 0.50, in 100 gms. of blood the amounts of lactate present in plasma and corpuscles will be 141.9 and 58.1 mg. respectively, assuming that the percentage of corpuscles in the whole blood is 45. The lactate ratio C/P in the oxygenated blood treated with lactic acid is 0.36. Assuming the above figure for the percentage of the corpuscles in the blood, the amounts of lactate present in the plasma and the corpuscles of 100 gms. of blood will/

will be 154.5 and 45.6 mg. respectively. Therefore in the respiratory cycle of a fatigued subject there is a to and fro lactate shift of 12.6 mg. per 100 gms. of blood, or about 6.5 per cent of the amount of lactate present in the whole blood.

ANAEMIA.

It is clear that haemoglobin (as a buffer) is responsible for the concentration of lactate in the corpuscles. The corpuscles in the blood of primary anaemia patients, being ten per cent larger with colour index more than one - (corresponding ten per cent increase in haemoglobin) - may possibly be capable of buffering more lactic acid than the corpuscles in the blood of normal subjects. This may be the reason for the high lactate ratio C/P (1.0) observed in the blood of anaemic patients.

It was pointed out in an earlier section that during exercise when the concentration of lactate in blood increases to over 150 mg. per 100 cc., the concentration of lactate in the corpuscles is neither 30 nor 36 per cent, as when sodium lactate and lactic acid respectively are added to blood, but is nearly 50 per cent of the concentration in plasma. This affords further support for the view that lactic acid, and not its salts, is discharged by the muscles into the blood. Therefore, as already shown (page 75) the corpuscles show a relatively higher concentration than that which would result if neutral lactate were discharged into the blood. It may be observed that lactic acid is discharged into the blood only in conditions of fatigue.

MUSCLE-SALINE AND BLOOD CORPUSCLES-SALINE SYSTEMS.

Lactate, in the systems blood corpuscles-saline or blood itself does not seem to be in simple osmotic equilibrium, as in the system muscle-saline. In the case of muscle-saline system:-

(a) The amount of labile base in combination with proteins in muscle (B.Pr) may be small compared to that in combination with haemoglobin in cells (B.Hb.) Hence change in pressure of carbon-dioxide may not alter the concentrations of lactate in muscle or the surrounding fluid, i.e. there is no to and fro lactate shift. For example, in the case of resting muscle, air, and in fatigued muscle, nitrogen, were used to stir the fluid, and yet no difference in the equilibrium concentration was observed, (except that due to the increase in the concentration of lactate in fatigued muscle).

(b) In the experiments to which reference has been made in this paper there was no to and fro transference of water in the muscle-saline system as in blood corpuscles-saline or blood itself. Therefore the muscle-saline system cannot be compared with the systems blood corpuscles-saline or blood itself, without taking into consideration the two differences mentioned above. On account of the large amount of labile base liberated in the corpuscles, (when the pressure of carbon-dioxide is increased), there is a shift/

shift of lactate (and chloride) into the cells to restore equilibrium; as this alters the osmotic equilibrium there is a transference of water to restore the latter. Thus the entire mechanism of osmotic equilibrium in blood system is different from and more complicated than, the simple osmotic equilibrium in muscle-saline system.

SUMMARY AND CONCLUSIONS.

Clausen's method modified by Cotonio, Friedmann and Shaffer, without aeration (Meyerhof) is applied for estimation of lactate in blood corpuscles, and found to yield 99 per cent of the lactate added to them. The presence of anticoagulant in the blood does not interfere with the almost complete recovery of lactate; the protein coagulum does not adsorb lactate.

The concentrations of lactate in the blood of rabbits, resting cats, horses and human subjects are 28, 20, 14 and 11 mg. per 100 gms. respectively. The ratio of concentration of lactate in the corpuscles to that in plasma in the blood of resting animals and men is 0.75, (when the concentrations are expressed in mg. per 100 gms).

There is a lactate shift from the plasma into the corpuscles, on equilibrating blood with a high pressure of carbon-dioxide, and from the corpuscles into the plasma on removal of carbon dioxide from the blood. The lactate shift into the corpuscles is more in reduced blood than in oxygenated blood. The lactate ratio C/P in venous blood is higher than in the oxygenated blood.

The/

The ratio of concentration of lactate in the corpuscles to that in plasma in the blood of fatigued cats and human subjects is 0.50. The fall in the lactate ratio C/P from 0.75 (in the blood of resting animals and men) to 0.50 (in the blood of fatigued animals and men) is associated with the increase in the concentration of lactate in the whole blood,as a result of exercise. The reverse effect, a progressive rise in the lactate ratio C/P, is noticed as the concentration of lactate in the blood of the fatigued subject decreases, after the cessation of exercise.

Similarly the ratio of concentration of lactate in the corpuscles to that in serum (defibrinated blood) falls steadily as the concentration of lactate in the blood is increased by the addition of lactate or lactic acid to blood; the same effect is observed by allowing glycolysis to take place in the blood. The ratio of concentration of lactate in the corpuscles to that in serum is 0.30 in the case of neutral salt sodium lactate and 0.36 in the case of lactic acid. The higher lactate ratio C/S in the case of lactic acid is due to its acid properties.

If the diffusion of lactate from the corpuscles into saline is allowed to take place, the ratio of concentration of lactate in the corpuscles to that in saline is the same as in the case of blood containing a low concentration of lactate. If the corpuscles are suspended/

suspended in Ringer Locke solution containing a high concentration of lactate, the ratio of concentration of lactate in the corpuscles to that in saline corresponds to the values obtained in the case of blood to which sodium lactate is added. The equilibrium condition in the blood corpuscles-saline system is the same in blood itself and that the serum has no selective property towards lactate.

The temperature coefficient for the lactate ratio C/P is almost negligible.

The low lactate ratio C/P in the blood of fatigued subjects is not due to the presence of indiffusible "pseudo-lactate" in the corpuscles, but due to the increase in the buffers in the plasma and to the increased concentration of bicarbonate and chloride in the corpuscles. The increased concentration of lactate in the plasma may be necessary for the maintenance of equilibrium between the plasma and the corpuscles which may have a higher osmotic pressure in conditions of fatigue. The apparent concentration of lactate in the resting frog muscle judged by its diffusion into Ringer is 20 mg. per 100 gm. As the muscle contains 80 per cent of water, the concentration of lactate in the resting frog muscle is 15 mg. per 100 gm. The apparent concentration of lactate in the fatigued frog muscle is 240 mg. per 100 gms. which gives a value of 192 mg. per 100 gm. for the concentration of lactate in the fatigued muscle.

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